SUPPORT FOR THE AMENDMENT

Support for the amendment to claim 1 is found in claim 7 as originally presented and on page 6, lines 9 and 13-20 of the specification. Support for the amendments to claims 3, 4, 8, and 20 is found on page 6, lines 14-20 of the specification. Support for claims 21, 22, 32, 33, 43 and 44 is found on page 7, lines 4-6 of the specification. Support for claims 24 and 35 is found in claim 2 as originally presented. Support for claims 25 and 36 is found in claim 3 as originally presented. Support for claims 26 and 37 is found in claim 4 as originally presented. Support for claims 27 and 38 is found in claim 5 as originally presented. Support for claims 28 and 39 is found in claim 8 as originally presented. Support for claims 29 and 40 is found in claim 12 as originally presented. Support for claims 30 and 41 is found in claim 13 as originally presented. Support for claims 31 and 42 is found in claim 14 as originally presented. Claims 9-11 and 15-19 have been canceled without prejudice to their prosecution in a continuation or divisional application.

No new matter would be added to this application by entry of this amendment.

Upon entry of this amendment claims 1-5, 8, 12-14 and 20-44 will now be active in this application with claims 1-5, 8, 12-14 and 21-44 being under active consideration.

REQUEST FOR RECONSIDERATION

The present invention is directed to a method of regulating autonomic nerve activity. Stimulation of parasympathetic activity over sympathetic activity is believed to reduce stress and calm aggravated mental states, inducing sleep. Oral and percutaneous administration of active ingredients as well as fragrances have been used to improve sleep induction. Some fragrances have been identified as disagreeable or irritating and accordingly new methods for regulating autonomic nerve activity are sought.

The present invention addresses this problem by providing a method for regulating autonomic nerve activity such as by increasing all ECG R-R interval, or decreasing systolic and/or diastolic blood pressure, by administering **by inhalation**, a terpene alcohol having a boiling point of at least 200°C, and having an odor below a detectable threshold. Applicants have discovered that administration of such terpene alcohols by inhalation is effective for regulating autonomic nerve activity. Such a method is no where disclosed or suggested in the cited prior art of record.

The rejections of Claims 1-14 under 35 U.S.C. § 103 over Swada and Binet et al. are respectfully traversed.

Neither of these references disclose or suggest a method of regulating autonomic nerve activity by administrating by inhalation a terpene alcohol.

Binet describes an investigation into the psychosedative and spasmolytic results by **orally** or **intravenously** administering synthetic farnesol. The reference neither discloses nor suggests a method of administration by inhalation.

Sawada et al. investigates the physiological effects of volatile components in forest, such as monoterpenes. No mode of administration is disclosed by this reference and accordingly a method of regulating autonomic nerve activity by administration by inhalation can not be suggested by this reference.

In contrast, the present invention is directed to a method for regulating autonomic nerve activity, in which a terpene alcohol is administered by inhalation. Applicants note that the claims have been amended to recite the limitation of claim 7, in that the alcohol is administered by inhalation.

As the cited references fail to disclose or suggest a method in which a terpene alcohol is administered by inhalation, the present invention is clearly not obvious from these

references and accordingly withdrawal of the rejections under 35 U.S.C. § 103 is respectfully requested.

The rejection of Claims 1-14 under 35 U.S.C. § 112, first paragraph and the objection to the specification are respectfully traversed.

Applicants respectfully submit that the claimed invention described in sufficient detail enable one of ordinary skill in the art to practice the claimed invention without undue experimentation. What appears to be at issue is the Examiner's belief that the claims merely recite the use of any composition possessing a "odor below a detectable threshold". Applicants respectfully submit that the claimed method is more significantly limited than described in the Official Action and accordingly the claimed invention may be practiced by one of ordinary skill in the art without undue experimentation.

Applicants note, the claims are defined by administration of a class of compounds of terpene alcohols. Terpene alcohols are alcohols of terpene compounds which are constructed of multiples of the 5-carbon hydrocarbon isoprene (2-methyl-1,3-butadiene), terpene compounds containing two isoprene units being called monoterpenes, containing three isoprene units being called sesquiterpenes, and containing 4, 6 and 8 units called diterpenes, triterpenes and tetraterpenes. (See attached passage from *Biochemistry, second edition* by A. L. Lehninger). Applicants note, that the claims have been amended to recite the genus of terpene alcohols based on the Examiner's recognition that the compounds on page 6 includes mono and di-terpene compounds. Applicants have corrected an obvious typographical error, the existence of and appropriate correction thereof being clear to those of ordinary skill in the art *In re Oda*, 170 USPQ 268 (CCPA (1971)). Accordingly, the claims are fundamentally limited to alcohol of terpene compounds.

Secondly, the claimed method is limited in that the terpene alcohol has a boiling point of at least 250°C. Such a recitation further limits the scope of the claimed compounds of

terpene alcohols in a very quantifiable manner. Terpene alcohol compounds in which the boiling point is below 250° are outside of the scope of the claims.

Further, the claimed method is limited to the use of terpene alcohol compounds having an odor below a detectable threshold. Having "an odor substantially below the detectable threshold" is defined on page 7, lines 4-6 of the specification. As such, the claimed method is believed to be sufficient described to enable those of ordinary skill in the art to practice the claimed invention without undue experimentation.

While the Examiner notes that a finite number of examples are set forth in the specification, such finite identification is not *per se* a failure to enable the invention without undue experimentation. Applicants note that all patent specification, which contain examples, only contain a finite number of examples.

However, Applicants note that they have presented working examples and the absence of an infinite number of disclosed compositions does not *per se* fail to provide sufficient working examples. As Applicants have provided working examples as well as an exemplary list of suitable compounds, defining a class of compounds quite narrow in terms of the chemical structure and physical properties thereof, the claimed invention is clearly enabled to those of ordinary skill in the art without undue experimentation.

Moreover, the burden is on the Patent Office to provide reasons based on scientific principles, to doubt the objective enablement of Applicant's claimed invention. Applicant's disclosure must be taken as in compliance with the enabling requirement under 35 USC 112, first paragraph, unless, there is reason to doubt the objective truth of the statements contained therein. (In re Marzocchi, 169 USPQ 367, 369 (CCPA 1971)). In the absence of any reasons provided by the Examiner, withdrawal of the rejection under 35 USC 112, first paragraph is respectfully requested.

Accordingly withdrawal of the rejection under 35 U.S.C. § 112, first paragraph is respectfully requested.

The rejection of claims 1-14 under 35 U.S.C. § 112, second paragraph has been obviated by appropriate amendment.

Applicants have now amended the method claims to recite the specific function of increasing ECG R-R interval, decreasing systolic blood pressure as well as decreasing diastolic blood pressure. The metes and bounds of the claimed invention are clear in view of Applicants' amendment. The attached preprint of Dayawansa et al. from *Autonomic Neuroscience: Basic and Clinical* 477, (2003) identifies the relationship between increasing an ECG R-R interval, decreasing systolic blood pressure, and decreasing diastolic blood pressure with autonomic responses. The attached abstracts of Malliani et al. from *Circulation* **(1991 Aug)** 84 (2) 482-92 and Pagani et al. from *Circulation Research* **(1986 Aug)** 59 (2) 178-93 describe relationships between heart rate activity and vagal or sympathetic activity. Withdrawal of the rejection under 35 U.S.C. §112 second paragraph is respectfully requested.

The rejection of Claim 3 under 35 U.S.C. § 112, second paragraph has been obviated by appropriate amendment.

As noted by the Examiner, some of the enumerated compounds fall outside of the meets and bounds of sesquiterpene alcohol and accordingly Applicants have now amended the claims to recite "terpene alcohol", consistent with the genus of compounds described. Applicants have corrected an obvious typographical error, the existence of and appropriate correction thereof being clear to those of ordinary skill in the art *In re Oda*, 170 USPQ 268 (CCPA (1971)). Accordingly withdrawal of this ground of rejection is respectfully requested.

Applicants submit that this application is now in condition for allowance and early notification of such action is earnestly solicited.

Respectfully submitted,

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SECOND EDITION

THE MOLECULAR BASIS

OF CELL STRUCTURE AND FUNCTION

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There are two major classes of nonsaponifiable lipids, the *terpenes* and the *steroids*. Although it is convenient to consider them as two distinct classes, they are closely related structurally, since both ultimately derive from five-carbon building blocks.

Terpenes

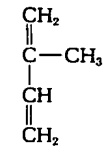
Terpenes are constructed of multiples of the five-carbon hydrocarbon *isoprene* (2-methyl-1,3-butadiene) (Figure 11-18). Terpenes containing two isoprene units are called *monoterpenes*, those containing three isoprene units are called *sesquiterpenes*, and those containing four, six, and eight units are called *diterpenes*, *triterpenes*, and *tetraterpenes*, respectively. Terpenes may be either linear or cyclic molecules; some terpenes contain structures of both types. The successive isoprene units of terpenes are usually linked in a head-to-tail arrangement, particularly in the linear segments, but sometimes the isoprene units are in tail-to-tail arrangement. The double bonds in the linear segments of most terpenes are in the stable trans configuration, but in *11-cis-retinal* (page 354), a derivative of vitamin A functioning in vision, one double bond is cis.

Of the very large number of terpenes identified in plants, many have characteristic odors or flavors and are major components of essential oils derived from such plants. Thus the monoterpenes *geraniol*, *limonene*, *menthol*, *pinene*, *camphor*, and *carvone* are major components of oil of geranium, lemon oil, mint oil, turpentine, camphor oil, and caraway oil, respectively. *Farnesol* is an example of a sesquiterpene. The diterpenes include *phytol*, a linear terpenoid alcohol, which is a component of the photosynthetic pigment chlorophyll (page 595). The triterpenes include *squalene*, an important precursor in the biosynthesis of cholesterol. Other higher terpenes include the *carotenoids*, a class of tetraterpene hydrocarbons and their oxygen-containing derivatives in which the head-to-tail arrangement of the isoprene units is characteristically reversed at the center of the molecule (Figure 11-19). An important carotenoid is *β-carotene*, the hydrocarbon precursor of vitamin A. Natural rubber and gutta-percha are *polyterpenes;* they consist of long hydrocarbon chains containing hundreds of isoprene units in regular linear order.

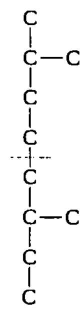
Among the most important terpenes are three members of the group of *fat-soluble vitamins*, namely, vitamins A, E, and K. Although these substances, which are required in trace amounts in the diet of mammals, may be classified among the lipids, their biological functions are so distinctive that their structure and function will be considered separately in Chapter 13, page 351.

Another important class of terpenes is represented by the *polyprenols*, long-chain linear polyisoprenoid compounds with a terminal primary alcohol group. The most important of these is *undecaprenyl alcohol*, also called *bactoprenol*, which contains 11 isoprene units and thus has 55 carbon atoms (Figure 11-19). *Dolichol* is the corresponding analog in

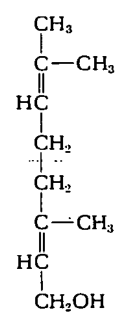
Figure 11-18
Isoprene units in the structur... ple terpenes.



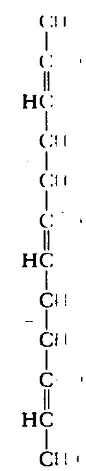
Isoprene



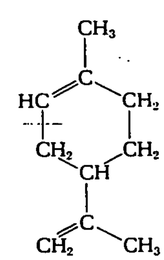
Head-to-tail or regular arrangement of isoprene units



Geraniol, a linear monoterpene



Farn... linear ses...



Limonene, a cyclic monoterpene

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Autonomic responses during inhalation of natural fragrance of "Cedrol" in humans

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Abstract

It is well known that odors affect behaviors and autonomic functions. Previous studies reported that some compounds in cedar wood essence induced behavioral changes including sedative effects. In the present study, we analyzed cardiovascular and respiratory functions while subjects were inhaling fumes of pure compound (Cedrol) which was extracted from cedar wood oil.

Vaporized Cedrol (14.2 ± 1.7 μg/l, 5 l/min) and blank air (5 l/min) were presented to healthy human subjects ($n=26$) via a face mask, while ECGs, heart rate (HR), systolic blood pressure (SBP), diastolic BP (DBP), and respiratory rates (RR) were monitored. Statistical analyses indicated that exposure to Cedrol significantly decreased HR, SBP, and DBP compared to blank air while it increased baroreceptor sensitivity. Furthermore, respiratory rate was reduced during exposure to Cedrol. These results, along with the previous studies reporting close relationship between respiratory and cardiovascular functions, suggest that these changes in respiratory functions were consistent with above cardiovascular alterations.

Spectral analysis of HR variability indicated an increase in high frequency (HF) component (index of parasympathetic activity), and a decrease in ratio of low frequency to high frequency components (LF/HF) (index of sympathovagal balance) during Cedrol inhalation. Furthermore, Cedrol inhalation significantly decreased LF components of both SBP and DBP variability, which reflected vasomotor sympathetic activity. Taken together, these patterns of changes in the autonomic parameters indicated that Cedrol inhalation induced an increase in parasympathetic activity and a reduction in sympathetic activity, consistent with the idea of a relaxant effect of Cedrol.

Keywords: Cedrol; Autonomic nervous system; Blood pressure; Heart rate; Baroreceptor sensitivity; Respiration

1. Introduction

It is well known that olfaction has a powerful influence on behaviors and physiological functions in mammals, and relationship between smell sensation and autonomic changes have been studied extensively. It is reported that odorants modulated retrieval of affective memory (Ehrlichman and Halpern, 1988), and induced heart acceleration (Bensafi et al., 2002) and changes in voice pitch (Millot and Brand, 2001). Some smells also induced autonomic changes compatible with autonomic relaxation (Alaoui-Ismaili et al., 1997; Nagai et al., 2000) and excitation (Brauchli et al., 1995; Alaoui-Ismaili et al., 1997), and autonomic changes compatible with basic emotions (Vernet-Maury et al., 1999). The authors in above studies attributed these autonomic changes to preference to the smells, associated behavioral changes and biological components of the smells. It has been demonstrated that the olfactory system has massive (direct or indirect) anatomical connections to the limbic system and hypothalamus (Ongur and Price, 2000). These neural circuits provide a link between olfactory sensation and autonomic responses.

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Cedrol is a crystalline natural substance derived from cedar wood (Juniperus Virginia) oil. Cedrol or cedar wood oil, which diffuses a week aroma, has been commonly used as an ingredient of shampoo, washing soap, facial soap, essence, etc. to enhance other fragrances. It has been reported that exposure to cedar wood oil increased Fos expression in the olfactory system (main olfactory bulb, anterior olfactory nucleus, piriform cortex, etc.) and the limbic system (amygdala, infralimbic cortex, entorhinal cortex, etc.) in rats (Amir et al., 1999a; Funk and Amir, 2000). Furthermore, exposure to extracted cedar essence increased amount of non-rapid eye movement (NREM) sleep in rats, decreased spontaneous activity and amount of wake in rats, and decreased NREM sleep latency in humans (Sano et al., 1998), while exposure to cedar wood oil enhanced photic resetting of the circadian clock in rats (Amir et al., 1999b). These results suggest that some compounds in cedar wood essence such as Cedrol cause sedation and suppression of sympathetic activity. However, physiological changes in humans during exposure to pure compounds included in cedar wood essence have not been reported.

The aim of the present study was to investigate effects of Cedrol fumes on human autonomic functions using non-invasive methods with spectral data to evaluate peripheral autonomic nerve activity. Reliability of spectral data in assessing autonomic status has been reported; high frequency (HF) component of heart rate variability (HRV) reflects activity of vagal parasympathetic system (Akselrod et al., 1981), and its low frequency (LF) component includes both sympathetic and parasympathetic activities (Parati et al., 1995). LF/HF ratio of the HRV denotes the balance between sympathetic and parasympathetic nervous system (Pagani et al., 1986; Hayano et al., 1991; Montano et al., 1994). LF power spectrum of blood pressure (BP) is an indirect measure of vasomotor sympathetic activity (Akselrod et al., 1985). Baroreceptor sensitivity, which is associated with BP and parasympathetic activity, was estimated using spectral data of R–R interval of ECG and systolic BP (Malliani et al., 1991).

2. Materials and methods

2.1. Subjects and materials

Healthy 26 (male, $n=10$; non-pregnant female, $n=16$) Japanese subjects (mean age, 24 years) were used. They were non-smokers and none of them had any olfactory problems nor were they on any form of medication at the time of the experiment. All experimental procedures were carried out pertaining to the ethics code of our institution with adequate understanding and consent of the subjects.

Subjects were asked to refrain from foods and drinks that would affect olfactory functions (alcohol, coffee, chewing gum, etc.) for 24 h, and to take adequate rest and sleep on the previous day. They were asked to report to the lab at 8.00 AM after 4 h fast before any form of physical excursion. Before the experiment, their physical conditions were assessed and all subjects were suitable for the experiment.

As blank air (control), charcoal (Charcoal activated granular, Wako, Japan)-filtered room air was used. In test, crystalline natural pure compound of Cedrol "[3R-(3α, 3aβ, 6α, 7β, 8aα)]-octahydro-3, 6, 8, 8-tetramethyl-1H-3a, 7-methanoazulen-6-ol)" (KAO Cosmetic Research Lab., Tokyo, Japan) was vaporized (see below in detail) and used.

2.2. Experimental setup and monitoring methods

Subjects were comfortably seated on an electrically driven reclining chair (chair with adjustments used for external dialysis) in a lit quiet 3 × 3 × 5-m room with an exhaust fan to re-ventilate the room air continuously. The inclination of upper body was set at 25° from the horizontal plane while legs were kept in horizontal plane using the chair adjustments. A previous study reported that heart rate variability of subjects was similar to that in supine position when passive tilt of the subjects was less than 30° (Montano et al., 1994). The left arm was kept at the level of the right atrium, and a noninvasive tonometric BP transducer, connected to a BP monitor (SA-250 EMC, Jentow CS, Colin, Japan), was strapped over the left radial artery. The sphygmomanometer cuff of an oscillometric BP recording device for calibration of the tonometric transducer was attached to the upper left arm. Surface electrodes were attached to the chest for recording of the electrocardiogram (ECG). Respiratory cycle was monitored via a respiratory thermo-sensor attached below the nostril (Thermal sensor, Nihon Kohden, Japan). Respiratory frequency was measured by manually counting the number of peaks per recording under visual inspection. We used the thermo-sensor, instead of thermal dissipation technique, to give optimum smell sensation to the subjects without undue uneasiness in seated position and to maintain a normal pattern of respiration without interference. All of these data were digitized at 500 Hz, displayed on a polygraph system, and stored on a hard disk (EEG-1100, Nihon Kohden).

Odorized and blank air was presented using an olfactometer with separate Teflon-coated tubing and face mask systems to prevent deposition of Cedrol particles. Charcoal-filtered room air was passed through a stainless steel container with a warmer to vaporize Cedrol at 90 °C, and presented to the subjects in constant concentration (14.2 ± 1.7 μg/l; 64.0 ± 7.7 10^{-9} M) at constant velocity (5.0 l/min) via a face mask. Blank air was similarly presented at 5.0 l/min except passing through the stainless steel container for Cedrol. Output temperature of both odorized and blank air was monitored via an air flow thermo-sensor (KP 15097, Okazaki, Japan) at the end of the tubing near the face mask, and was kept constant at 27.0 ± 0.5 °C.

2.3. Experimental procedure

The subjects were instructed to relax and breathe normally, and that their autonomic functions would be measured during inhalation of odorized or blank air. They were told which stimulus air would be presented to them before the experiment to avoid undue adjustment of respiration, and were required to raise their right thumb when they detected smell. After setting up a face mask, various probes and electrodes on the subject, data were recorded for 5–10 min without any stimulus to check the monitoring devices and to accustom the subjects to the new environment. Then, to avoid possible prolonged effects of Cedrol, blank air was firstly presented for 10 min followed by odorized air (Cedrol) for 10 min with an inter-stimulus interval of 8 min. The BP measurement was calibrated before each stimulus presentation. For the control experiment, blank air was presented twice in the same way as the Cedrol experiment; for 10 min each with an interval of 8 min. This experiment was performed to verify the effects were due to Cedrol itself or to relaxation over time.

2.4. Data analysis

Ten-minute recordings during presentation of blank and odorized air were taken for analysis. Beat to beat (R–R) intervals of heart rate were measured by detecting a peak of QRS waves of the ECGs, and HR was estimated from the R–R intervals. Power spectral analyses of variability of these cardiovascular parameters (i.e., heart rate (HR), systolic blood pressure (SBP), diastolic BP (DBP)) were performed with the fast Fourier transformation technique (FFT; 2048 points) through a Hamming window. Balance between sympathetic and parasympathetic outflow was estimated as a ratio of low (0.03–0.12 or 0.03–0.15 Hz) and high (0.12–0.5 or 0.15–0.5 Hz)-frequency components of the power spectrum of the HR variability (LFHRV/HFHRV), and parasympathetic outflow as the high-frequency component (HRV-HF) (Pagani et al., 1986; Malliani et al., 1991). Since the boundary between the LF and HF components was previously set at 0.12 Hz (Stanley et al., 1996) or 0.15 Hz (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), we calculated these components in both conditions. We also estimated LF components derived from the power spectral analysis of the SBP and DBP variability (SBPV-LF, DBPV-LF) which reflected muscle sympathetic activity (Goso et al., 1999). Furthermore, a ratio of SBPV-LF to HRV-HF (LFSBPV/HFHRV) was assessed as sympathovagal balance.

Arterial baroreflex sensitivity (BRS) was estimated by spectral analysis using the "α-index" method (Akselrod et al., 1985; Pagani et al., 1988; Malliani et al., 1991). This sensitivity equals the gain (α-index) of the transfer function between the oscillations of SBP and HR in the LF range (Robbe et al., 1987). The BRS (α-index) was obtained with formula:

$$\mathrm{BRS} = [P_{\mathrm{R-R\ (LF)}}/P_{\mathrm{SBP\ (LF)}}]^{1/2}$$

where $P_{\mathrm{R-R\ (LF)}}$ and $P_{\mathrm{SBP\ (LF)}}$ represent low frequency components of the spectral power of the R–R interval (ECG) and of the SBP, respectively. The validity of this calculation requires that the squared coherence between the two variability signals is greater than 0.5 (Pagani et al., 1988; Lucini et al., 1994).

Respiratory rates (RR) were calculated from temperature curve at the nostril. All data were expressed as mean ± S.E.M. Since the control experiment indicated that there were no significant differences in the autonomic parameters between the 1st and 2nd presentation of blank air (see Results), the data in each parameter in the Cedrol experiment were compared between blank air (1st presentation) and Cedrol (2nd presentation) conditions by a paired *t*-test. A value of $p < 0.05$ was defined as statistically significant.

3. Results

3.1. Representative data of individual cardiovascular functions

Data for the control experiment were taken from the nine subjects. The results in the control experiment are shown in Table 1. There were no significant differences in the autonomic parameters between the 1st and 2nd presentation of blank air (paired *t*-test, $p > 0.05$).

Data for the Cedrol experiment were taken from the 26 subjects. However, data from 3 of the 26 subjects were

Table 1
Effects of stimulus sequence on the autonomic functions in the control experiment

Measure	Blank air (1st) ($n=9$)	Blank air (2nd) ($n=9$)	p
HR (beats/min)	73.6 ± 3.4	70.8 ± 3.2	>0.05
SBP (mm Hg)	115.7 ± 4.6	115.9 ± 4.0	>0.05
DBP (mm Hg)	64.5 ± 2.5	63.3 ± 3.4	>0.05
RR (Hz)	0.248 ± 0.026	0.281 ± 0.022	>0.05
HRV-HF [(beats/min)^2/Hz]	0.98 ± 0.14	1.28 ± 0.25	>0.05
LFHRV/HFHRV	1.38 ± 0.63	0.89 ± 0.19	>0.05
SBPV-LF [(mm Hg)^2/Hz]	1.30 ± 0.32	1.20 ± 0.27	>0.05
DBPV-LF [(mm Hg)^2/Hz]	1.77 ± 0.84	1.36 ± 0.27	>0.05
LFSBPV/HFHRV [(mm Hg)^2/(beats/min)^2]	1.74 ± 0.41	1.17 ± 0.38	>0.05
BRS (ms/mm Hg)	11.7 ± 2.1	12.6 ± 2.3	>0.05

The boundary between the LF and HF components was set at 0.15 Hz. The values are presented as mean ± S.E.M. The "*p*" value indicated statistical significance by paired *t*-test. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RR, respiration rate; HRV-HF, high frequency component of the HR variability; SBPV-LF, low frequency component of SBP variability; LFSBPV/HFHRV, ratio of SBPV-LF to HRV-HF; BRS, baroreflex sensitivity.

discarded; contamination of noise artifacts by body motion for 2 subjects and discontinuation of the experiment due to a dislike of the smell for 1 subject. The data from the remaining 23 subjects were applied to the following analyses. All 26 subjects detected the smell of Cedrol. Fig. 1A shows examples of HR changes during inhalation of blank air (a) and Cedrol (b). HR was around 70–80 beats/min during exposure to blank air while HR gradually decreased during Cedrol inhalation. It is noted that HR fluctuated more slowly during exposure to Cedrol. Fig. 1B shows results of spectral analysis of above data of HR variability (HRV) during exposure to blank air (a) and Cedrol (b). The HF component of HR variability increased from 1.83 to 3.46 (beats/min)^2/Hz, while LFHRV/HFHRV ratio decreased from 0.24 to 0.18 by Cedrol inhalation. This suggested an enhancement of parasympathetic activity, compared with sympathetic activity, during Cedrol inhalation.

Fig. 2A shows examples of SBP changes of the same subject shown in Fig. 1 during inhalation of blank air (a) and Cedrol (b). The SBP was around 110–120 mm Hg during exposure to blank air, while it dropped to 100–110 mm Hg during Cedrol exposure. It is noted that SBP fluctuated more slowly during exposure to Cedrol. Fig. 2B shows results of spectral analysis of SBP variability noted above during exposure to blank air (a) and Cedrol (b). The LF component decreased from 1.40 to 1.25 (mm Hg)^2/Hz, suggesting suppression of sympathetic activity during Cedrol inhalation.

3.2. Comparison of cardiovascular functions

Table 2 shows statistical comparisons of HR, SBP, and DBP between blank air and Cedrol exposure using data of the 23 subjects. HR significantly decreased during Cedrol inhalation (paired t-test, $p<0.05$). Consistent with this HR change, SBP significantly decreased during Cedrol inhalation (paired t-test, $p<0.05$), and DBP significantly decreased during Cedrol inhalation (paired t-test, $p<0.05$).

Statistical comparisons of activity of the peripheral autonomic nervous system are shown in Table 2. Autonomic nervous activity was estimated from spectral analysis of HR, SBP and DBP variability between blank air and Cedrol exposure, where the boundary between the LF and HF components was set at 0.12 Hz in Table 2. The HF component of HRV (HRV-HF), which is an index of parasympathetic activity significantly increased during Cedrol inhalation (paired t-test, $p<0.05$). A ratio of HRV-LF to HRV-HF (LFHRV/HFHRV) which is an index of the balance between sympathetic and parasympathetic activity significantly decreased during Cedrol inhalation (paired t-test, $p<0.05$). Furthermore, a ratio of SBPV-LF to HRV-HF (LFSBPV/HFHRV), which is suggested as a better reflection of sympathovagal balance, significantly decreased during Cedrol inhalation (paired t-test, $p<0.05$). Consistent with the data derived from HR variability, LF components of SBP and DBP variability, which are indices of the vasomotor sympathetic tone, displayed similar changes; these indices signifi-

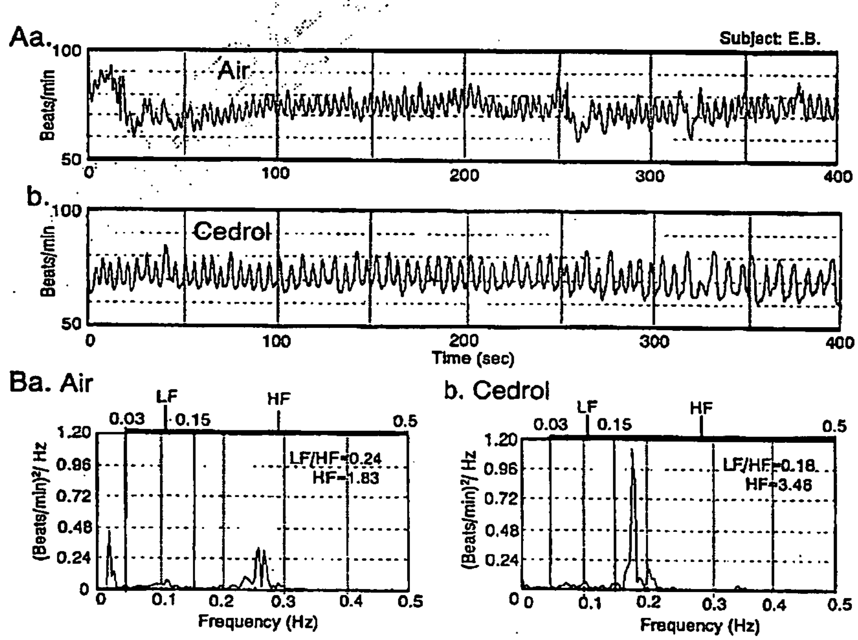

Fig. 1. Representative data of HR and HR variability during exposure to blank air and Cedrol. (A) Time course of HR changes during inhalation of blank air (a) and Cedrol (b). Note that HR gradually decreased and fluctuated more slowly during Cedrol inhalation. (B) Spectral analysis of HR variability shown in A during exposure to blank air (a) and Cedrol (b). Note that HF component of HR variability increased from 1.83 to 3.46 (beats/min)^2/Hz, while LF/HF ratio decreased from 0.24 to 0.18 by Cedrol inhalation.

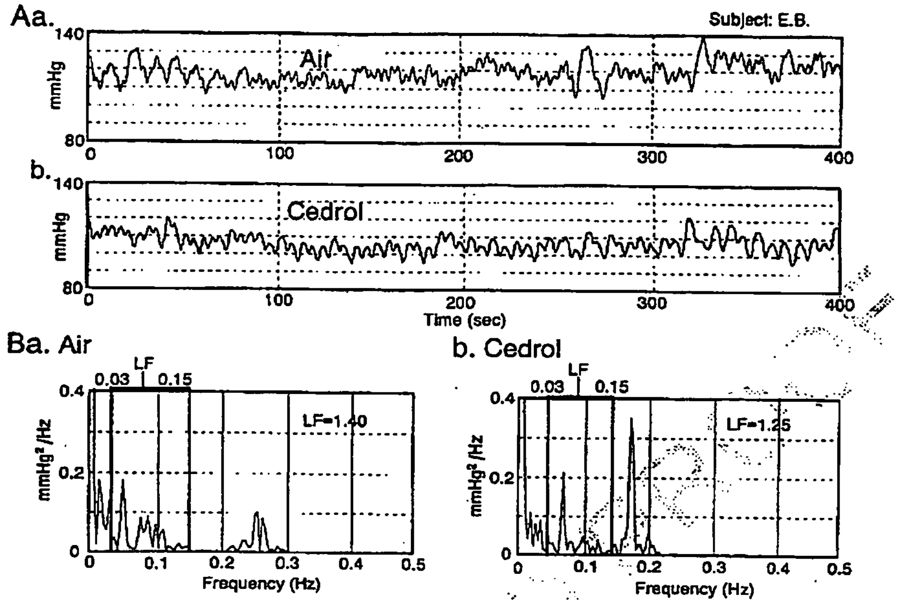

Fig. 2. Representative data of systolic BP (SBP) and SBP variability during exposure to blank air and Cedrol. (A) Time course of SBP changes of the same subject shown in Fig. 1 during inhalation of blank air (a) and Cedrol (b). Note that SBP decreased to 110–100 mm Hg during exposure to Cedrol (b). (B) Spectral analysis of SBP variability shown in A during exposure to blank air (a) and Cedrol (b). Note that LF component decreased from 1.40 to 1.25 (mm Hg)^2/Hz during inhalation of Cedrol.

cantly decreased during Cedrol inhalation in both SBP and DBP variability (paired t-test, $p<0.05$). The same analyses were performed except that the boundary between the LF and HF components was set at 0.15 Hz in Table 3. Essentially the same results were obtained.

Results in Tables 2 and 3 show statistical comparison of ratios between LF components of HR and SBP variability (alpha index), which reflects baroreceptor sensitivity (BRS). Since the data from 3 of the 23 subjects showed the squared coherence less than 0.5, these data were discarded. In both results in Tables 2 and 3 where the boundary between the LF and HF components was set at 0.12 and 0.15 Hz, respectively, baroreceptor sensitivity significantly increased during Cedrol inhalation (paired t-test, $p<0.05$).

3.3. Comparison of respiratory functions

Table 2 shows statistical comparisons of respiratory frequency. Respiration rate (RR) significantly decreased

Table 2
Effects of Cedrol inhalation on the autonomic functions in the Cedrol experiment

Measure	Blank air (1st) ($n=23$)	Cedrol (2nd) ($n=23$)	p
HR (beats/min)	70.7 ± 1.9	68.7 ± 1.6	<0.05
SBP (mm Hg)	119.5 ± 3.2	112.8 ± 2.6	<0.05
DBP (mm Hg)	66.4 ± 2.1	62.8 ± 2.1	<0.05
RR (Hz)	0.250 ± 0.014	0.223 ± 0.011	<0.05
HRV-HF [(beats/min)^2/Hz]	1.17 ± 0.18	1.57 ± 0.25	<0.05
LFHRV/HFHRV	0.795 ± 0.149	0.509 ± 0.102	<0.05
SBPV-LF [(mm Hg)^2/Hz]	1.48 ± 0.33	0.87 ± 0.18	<0.05
DBPV-LF [(mm Hg)^2/Hz]	1.50 ± 0.37	0.76 ± 0.14	<0.05
LFSBPV/HFHRV [(mm Hg)^2/(beats/min)^2]	1.91 ± 0.65	0.67 ± 0.14	<0.05
BRS (ms/mm Hg) ($n=20$)	11.5 ± 1.3	13.9 ± 1.3	<0.05

The boundary between the LF and HF components was set at 0.12 Hz.
The values are presented as mean ± S.E.M. Data from three subjects were discarded in the BRS since the coherence between the HR and SBP variability did not exceed 0.5 in these three subjects. For other descriptions, see Table 1.

Table 3
Effects of Cedrol inhalation on the autonomic functions in the Cedrol experiment

Measure	Blank air (1st) ($n=23$)	Cedrol (2nd) ($n=23$)	p
HRV-HF [(beats/min)^2/Hz]	0.95 ± 0.13	1.31 ± 0.23	<0.05
LFHRV/HFHRV	1.34 ± 0.31	0.85 ± 0.24	<0.05
SBPV-LF [(mm Hg)^2/Hz]	1.69 ± 0.37	0.94 ± 0.19	<0.05
DBPV-LF [(mm Hg)^2/Hz]	1.62 ± 0.40	0.83 ± 0.14	<0.05
LFSBPV/HFHRV [(mm Hg)^2/(beats/min)^2]	2.54 ± 0.86	0.87 ± 0.19	<0.05
BRS (ms/mm Hg) ($n=20$)	11.9 ± 1.4	14.8 ± 1.3	<0.05

The same data as those in Table 2 were analyzed, but the boundary between the LF and HF components was set at 0.15 Hz.
The values are presented as mean ± S.E.M. Data from three subjects were discarded in the BRS since the coherence between the HR and SBP variability did not exceed 0.5 in these three subjects. For other descriptions, see Table 1.

during Cedrol inhalation (paired t-test, $p<0.05$). This change in RR might affect cardiovascular functions since respiration is a powerful modulator of cardiovascular functions (Stanley et al., 1996; Bernardi et al., 2001) (see Discussion).

4. Discussion

4.1. Changes in cardiovascular functions during exposure to Cedrol

The results demonstrated a significant reduction in SBP, DBP, and HR during Cedrol inhalation. Spectral analysis of HR variability indicated a significant increase in HF component and reduction in both LFHRV/HFHRV and LFSBPV/HFHRV ratios during exposure to Cedrol. Furthermore, LF component of SBP and DBP variability significantly decreased during exposure to Cedrol. These changes in the cardiovascular parameters were consistent in terms of peripheral autonomic nerve activity, suggesting that exposure to Cedrol increased parasympathetic activity while it reduced sympathetic activity.

Baroreceptor sensitivity significantly increased during exposure to Cedrol. Consistent with the present results, increase in baroreceptor sensitivity was associated with increase in parasympathetic activity and reduction in sympathetic activity (Bernardi et al., 2002), and a reduction in BP (Conway et al., 1983). It has been reported that baroreceptor sensitivity decreased with increasing mental arousal (Conway et al., 1983), physical exercise (Bristow et al., 1971; Mancia et al., 1978), mental stimulation (Sleight et al., 1978), and emotional behavior (Hilton, 1975). These results, along with lesion studies (Verberne et al., 1987; Hoff et al., 1963; Miyajima and Bunag, 1985), suggest that the higher brain areas in the forebrain such as the medial, lateral prefrontal cortices, hypothalamus, and limbic system inhibit baroreflex. Afferent fibers from the baroreceptors project to the medulla, pons, and hypothalamus (Spyer, 1972) where the baroreflex arc might interact with the higher brain areas. Since Cedar wood oil activated the limbic system (see Introduction), Cedrol might release the baroreflex from the central inhibition through the limbic system.

4.2. Possible relations between respiratory and cardiovascular functions

The respiratory system has an intimate relationship with cardiovascular system (Shepherd, 1981; Malpas, 1998; Bernardi et al., 2001). Modulatory effects of respiration on HR variability and baroreceptor sensitivity have been reported (Bernardi et al., 2001, 2002). In healthy and heart failure subjects, muscle sympathetic nerve activity was associated with respiratory function; both increment in lung inflation and decrement in respiratory rate decreased muscle sympathetic nerve activity (Seals et al., 1990; Naughton et al., 1998; Goso et al., 2001). These results suggest that afferent fibers from lung stretch receptors modulated the respiratory and cardiovascular centers through Hernig-Breur reflex (lung inflation reflex) to reduce muscle sympathetic nerve activity (Goso et al., 2001), or that respiratory changes in BP stimulated baroreflex, which in turn modulated the central cardiovascular system (Bernardi et al., 2001). Furthermore, reduction in respiratory rate increased baroreceptor sensitivity and decreased SBP and DBP (Bernardi et al., 2002), and that baroreceptor sensitivity was inversely related to sympathetic activity (Somers et al., 1989a,b, 1991). This suggests that reduction in RR increased baroreceptor sensitivity in the present study.

The patterns of respiratory and cardiovascular changes during exposure to Cedrol in the present study were consistent with the previous studies noted above; a pattern of the present results suggests that the cardiovascular changes during exposure to Cedrol might be ascribed to respiratory alteration induced by exposure to Cedrol. However, direct relationships between the respiratory and cardiovascular changes were not analyzed in the present study. Further studies are required to elucidate direct effects of Cedrol on the cardiovascular system; it would be interesting to assess cardiovascular changes during exposure to Cedrol under metronomic breathing or controlled tidal volume.

4.3. Sites of Cedrol action

Human non-invasive studies reported that cerebral blood flow increased in the orbital and piriform cortices, amygdala as well as the hypothalamus during odor perception and judgment of odor intensity or pleasantness (Zatorre et al., 1992, 2000; Zald and Pardo, 2000). Animal studies indicated that presentation of Cedar wood oil induced activity increase in the olfactory system as well as various brain areas in the limbic system (Amir et al., 1999a; Funk and Amir, 2000). These olfaction-related areas send descending visceromotor outputs, and play an important role in autonomic functions (Ongur and Price, 2000). Cedrol might induce autonomic alteration through the central olfactory and limbic systems.

It has been reported that rats exposed to essential oil vapors displayed motility increment or decrement according to the type of the smells, and fragrance compounds were found in their serum (Buchabauer et al., 1993). These results suggest that Cedrol might directly act on the peripheral autonomic systems via a blood-borne rout. It is also possible that Cedrol directly acts on peripheral afferent fibers of the vagal nerve innervating in the respiratory system, which in turn might induce changes in the peripheral autonomic nervous system through the central nervous system. Although its mechanism(s) remain to be identified, it is unlikely for its action to be a simple vasodilator since compensatory physiological mechanisms such as HR increment were not observed during exposure to Cedrol. Further studies are necessary to elucidate whether a peripherally or

centrally mediated pathway or both the pathways are involved in the autonomic changes observed in the present study.

4.4. Conclusions

A previous study indicated that exposure to extracted cedar essence increased amount of non-rapid eye movement (NREM) sleep in rats, decreased spontaneous activity and amount of wake in rats, and decreased NREM sleep latency in humans (Sano et al., 1998). The non-invasive analyses of cardiovascular parameters demonstrated that exposure to Cedrol increased parasympathetic activity while it reduced sympathetic activity with concomitant alteration of respiratory functions in the present study. These results strongly suggest that Cedrol has a relaxant effect. However, the exact mechanisms of these effects remained to be elucidated although both centrally and peripherally mediated mechanisms were suggested. Finally, these changes in respiratory and cardiovascular changes induced by exposure to Cedrol might ameliorate autonomic disturbances in chronic heart failure (Bernardi et al., 2002).

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Power Spectral Analysis of Heart Rate and Arterial Pressure Variabilities as a Marker of Sympatho-Vagal Interaction in Man and Conscious Dog

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In 57 normal subjects (age 20–60 years), we analyzed the spontaneous beat-to-beat oscillation in R–R interval during control recumbent position, 90° upright tilt, controlled respiration (n = 16) and acute (n = 10) and chronic (n = 12) β-adrenergic receptor blockade. Automatic computer analysis provided the autoregressive power spectral density, as well as the number and relative power of the individual components. The power spectral density of R–R interval variability contained two major components in power, a high frequency at ~0.25 Hz and a low frequency at ~0.1 Hz, with a normalized low frequency : high frequency ratio of 3.6 ± 0.7. With tilt, the low-frequency component became largely predominant (90 ± 1%) with a low frequency : high frequency ratio of 21 ± 4. Acute β-adrenergic receptor blockade (0.2 mg/kg IV propranolol) increased variance at rest and markedly blunted the increase in low frequency and low frequency : high frequency ratio induced by tilt. Chronic β-adrenergic receptor blockade (0.6 mg/kg p.o. propranolol, t.i.d.), in addition, reduced low frequency and increased high frequency at rest, while limiting the low frequency : high frequency ratio increase produced by tilt. Controlled respiration produced at rest a marked increase in the high-frequency component, with a reduction of the low-frequency component and of the low frequency : high frequency ratio (0.7 ± 0.1); during tilt, the increase in the low frequency : high frequency ratio (8.3 ± 1.6) was significantly smaller. In seven additional subjects in whom direct high-fidelity arterial pressure was recorded, simultaneous R–R interval and arterial pressure variabilities were examined at rest and during tilt. Also, the power spectral density of arterial pressure variability contained two major components, with a relative low frequency : high frequency ratio at rest of 2.8 ± 0.7, which became 17 ± 5 with tilt. These power spectral density components were numerically similar to those observed in R–R variability. Thus, invasive and noninvasive studies provided similar results. More direct information on the role of cardiac sympathetic nerves on R–R and arterial pressure variabilities was derived from a group of experiments in conscious dogs before and after bilateral stellectomy. Under control conditions, high frequency was predominant and low frequency was very small or absent, owing to a predominant vagal tone. During a 9% decrease in arterial pressure obtained with IV nitroglycerin, there was a marked increase in low frequency, as a result of reflex sympathetic activation. Bilateral stellectomy prevented this low-frequency increase in R–R but not in arterial pressure autospectra, indicating that sympathetic nerves to the heart are instrumental in the genesis of low-frequency oscillations in R–R interval. (*Circulation Research* 1986;59:178–193)

The whole history of brain electrophysiology has proved that rhythms can often be markers of normal functional states such as wakefulness or sleep, or of abnormal states such as epilepsy. In the present context, the general hypothesis is that rhythmical beat-to-beat oscillations of one cardiovascular controlled variable might provide some criteria to interpret the complex interplay among the neural regulatory outflows. On the other hand, the existence, under stable conditions, of rhythmic fluctuations in cardiovascular variables such as heart rate or arterial pressure has been recognized for a long time.[1-7] In recent years, the possibility of quantifying these oscillations by using computer techniques, particularly on the variability of electrocardiographic R–R interval, has aroused a growing interest.[8-15]

Indeed, as instantaneous heart rate depends on the interaction between sympathetic and parasympathetic efferent activities and pacemaker properties, it has been suggested that this analysis could lead to a noninvasive assessment of the "tonic" autonomic regulation of heart frequency.[12] The nonrandom components of R–R variability usually are assessed with spectral techniques based on the fast Fourier transform, in spite of the consideration that the heart rate variability signal is not strictly periodical, as requested by the deterministic nature of the algorithm.[16]

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In conscious dogs and in man, a high-frequency component (~0.25 Hz) has been consistently found in the power spectrum and has been interpreted as a quantitative assessment of respiratory arrhythmia.[15] One or two low-frequency components have also been described, respectively, about 0.1 Hz and 0.03 Hz. The former of these, the so-called 10-second period rhythm, has been considered particularly interesting as it has the same frequency of the better known Mayer[5] waves.[17]

As to the neural mechanisms underlying these fluctuations, vagal efferent activity has been interpreted as being primarily responsible for the high-frequency component of heart rate variability on the basis of experiments on vagotomized decerebrated cats,[10] conscious dogs,[12] and man with muscarinic receptor blockade.[15]

Both vagal and sympathetic outflows were considered to determine the low-frequency components.[12,15]

The aim of this study on normal human subjects and conscious dogs was to assess the relative role of vagal and sympathetic activities in determining the variability in heart rate and arterial pressure at rest and during induced changes of autonomic regulation. A totally automatic computation of the autospectra and of their individual components was implemented, using an autoregressive modeling algorithm, in order to take advantage of its inherent better definition, compared to the more current spectral techniques.[18,19]

Subjects and Methods

Fifty-seven subjects without any clinically evident disease (20–60 years old) were used for this study. They were healthy volunteers, randomly selected from hospital staff, medical students, and their relatives. Seven additional subjects consulted our hypertension clinic and were eventually found to be normotensive in the hospital environment. These subjects underwent 24-hour continuous monitoring of electrocardiogram (ECG) and high-fidelity intraarterial systemic pressure recording[20] as part of their clinical workup for suspected hypertension.

All subjects included in this study had no signs of organ or systemic disease. Subjects smoking more than five cigarettes a day were excluded from the study. No one was taking any medication, and each subject was instructed to avoid beverages containing alcohol or caffeine after 10 pm of the day preceding the study and to come to the laboratory after a light breakfast. Studies were performed between 10:30 AM and 12:30 PM. All subjects were carefully instructed about the study, and all gave their informed consent.

Tilting

Subjects were placed on an electrically driven tilt table (provided with a mattress and a footrest) and connected to a conventional electrocardiographic amplifier (lead II, Cardioline) and FM tape recorder (Racal). After 15–30 minutes, allowed for stabilization, the ECG was continuously recorded for 30 minutes at rest, followed by 20 minutes after the subjects had been passively moved to an upright 90° position by electrically rotating the table. During tilting, the subjects rested on their feet, although they had been loosely strapped to the table to avoid accidental falls.

The arterial blood pressure was recorded at the beginning and at the end of "rest" and "tilt" periods using a conventional sphygmomanometer.

Reproducibility of the results was assessed by repeating the study in 10 subjects after a period of 1 week to 12 months. Additionally, in 4 subjects, a third study was performed after an additional 3–4 months.

The effects of β-adrenergic receptor blockade was assessed in two groups of experiments. In 10 subjects, after the baseline studies, the study was repeated on a later day after IV bolus of 0.2 mg/kg propranolol (Inderal, ICI) in order to block acutely β-adrenergic receptor-mediated effects (Table 1).

In 12 subjects, the study was repeated after 6 days of 0.6 mg/kg p.o. propranolol (Inderal, ICI) t.i.d. in order to block chronically β-adrenergic receptor mediated effects (Table 1).

Controlled Respiration

The effects of controlled respiration were tested in 16 subjects both at rest and during tilt. These patients were studied first while breathing spontaneously, and on a separate day while breathing at 20 acts/min, following a metronome. Respiratory movements were monitored with an impedance respirometer (Cardioline) and recorded on FM tape. Seven of these patients breathed through a mouthpiece connected to a spirometer (Jaeger) in order to quantify their tidal volume. All of these patients had been well acquainted with the laboratory through 1–3 training sessions, during which they learned to breathe quietly and to adjust their tidal volume to the increased respiratory rate. During spontaneous and controlled breathing, transcutaneous P_{CO_2} and P_{O_2} (Sensor Medics) were monitored in 5 patients.

Systemic Arterial Pressure (Invasive Studies)

In 7 additional subjects, a microminiature Millar tip pressure transducer (03 French) was inserted percutaneously into the radial artery of the nondominant arm with a Seldinger technique.

Calibrations of the transducer, both in absolute values of millimeters of mercury and millivolts, were performed just before the insertion of the catheter and at the end of the recording procedure. After suitable amplification, arterial blood pressure and ECG were continuously recorded at rest and during tilt on FM tape. These studies were the last part of a 24-hour protocol for suspected hypertension, based on a continuous ambulatory recording of direct high-fidelity arterial pressure and ECG.[20]

Animal Experiments

Twelve mongrel dogs (25–35 kg body weight) were used for this part of the study. They were lightly anesthetized with thiopental sodium (10 mg/kg, IV) (Farmotal, Farmitalia) and, after the skin had been infiltrated with 2% xylocaine (Byk Gulden), a cut-down was

TABLE 1. *Effects of Age and β-Adrenergic Blockade on the Response of R-R Interval Variability to Tilt*

	Arterial pressure				Low-frequency component		High-frequency component		
	Systolic (mm Hg)	Diastolic (mm Hg)	R-R interval (msec)	R-R variance ($msec^2$)	Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)	LF:HF ratio
A. Rest									
20–30 yr (*n* = 30)	116 ±2	73 ±2	834 ±34	4097 ±361*	58.2 ±3.3	0.11 ±0.01	24.0 ±2.0	0.24 ±0.02	3.62 ±0.70
30–45 yr (*n* = 10)	116 ±4	79 ±3	931 ±39	2581* ±356*	62.2 ±6.3	0.10 ±0.01	26.3 ±4.3	0.27 ±0.02	3.69 ±1.23
45–60 yr (*n* = 17)	118 ±3	78 ±2	926 ±27	1354 ±205	49.9 ±4.9	0.09 ±0.01	32.0 ±4.6	0.27 ±0.01	2.58 ±0.61
Acute β-adrenergic blockade, 20–30 yr (*n* = 10)	103 ±3†	70 ±3	1076 ±26†	8255 ±1676†	50.9 ±5.0	0.12 ±0.01	32.7 ±4.3	0.26 ±0.02	1.74 ±0.31
Chronic β-adrenergic blockade, 20–30 yr (*n* = 12)	106 ±2†	66 ±2†	1115 ±59†	7180 ±1712†	39.4 ±5.7†	0.12 ±0.01	53.4 ±5.4†*	0.26 ±0.02	1.07 ±0.35†
B. Tilt									
20–30 yr	111 ±1	79 ±2	687 ±14 ‡	2642 ±217 ‡	89.7 ‡ ±1.4	0.09 ±0.01	7.5 ±0.9 ‡	0.29 ±0.03	20.79 ±3.68‡
30–45 yr	109 ±4	78 ±3	719 ±44*‡	2160 ±253*	83.7 ±4.6*‡	0.09 ±0.01	11.4 ±2.8*‡	0.29 ±0.06	17.30 ±8.19‡
45–60 yr	113 ±3	77 ±2	767 ±24 ‡	1212 ±213	75.7 ±4.6 ‡	0.08 ±0.01	16.2 ±3.4 ‡	0.23 ±0.02	12.61 ±3.21‡
Acute β-adrenergic blockade, 20–30 yr	100 ±4†	72 ±4	929 ±24†‡	3009 ±361‡	74.1 ±7.0†‡	0.09 ±0.01	13.0 ±2.5‡	0.22 ±0.02	7.48 ±1.77†‡
Chronic β-adrenergic blockade, 20–30 yr	97 ±2†‡	69 ±4†	951 ±35†‡	4067 ±664†‡	66.7 ±5.2†‡	0.11 ±0.01	24.0 ±4.4†‡	0.22 ±0.02	3.78 ±0.93†‡

*Significantly different contrast ($p < 0.05$).
†Value during β-adrenergic blockade significantly different ($p < 0.05$) from value obtained in unblocked conditions in the young age group.
‡Value during tilt significantly different from value at rest ($p < 0.05$).

performed in the region of the femoral artery. A catheter was introduced into it, advanced to the aorta, and secured with a suture. The wound was closed, and the catheter was exteriorized at the base of the neck.

After baseline studies, 5 dogs underwent bilateral stellectomy. By sterile techniques, they were anesthetized with thiopental sodium (30 mg/kg, IV) followed by a continuous infusion of fentanyl citrate (6.2 μg/kg, IV) and droperidol (0.2 mg/kg, IV) (Leptofen, Carlo Erba). The animals were paralyzed with small (0.1 mg/kg, IV) intermittent doses of succinylcholine (Wellcome) and artificially ventilated with a positive-pressure pump (Harvard). A thoracotomy was performed in the fourth left intercostal space, the left stellate ganglion and its branches were excised, and the thoracotomy was repaired. Seven to 15 days later, under similar anesthesia and through a right thoracotomy in the fourth intercostal space, the right stellate ganglion was excised as well.

Aortic pressure was measured with the implanted catheter using a pressure transducer (Statham Instruments). Aortic mean pressure was obtained with an RC filter with a 2-second time constant. The electrocardiogram (lead II) was obtained with subcutaneous silver electrodes and an AC amplifier. Heart rate was monitored continuously with a cardiotachometer triggered by the R-wave.

Respiratory movements were monitored with a pneumatic belt connected to a pressure transducer (Statham Instruments). Data were recorded on a multichannel FM tape recorder (Racal Store 7) and played back on a direct-writing recorder (Brush Gould).

Experiments were performed after a postoperative period long enough to allow complete recovery of the dogs from the operation, as judged by their normal behavior, body temperature, and hematocrit. Five to 7 days usually were sufficient after the implantation of the pressure catheter, while 10–15 days were given after the thoracotomy. While the trained dogs were lying quietly on the recording table, aortic blood pressure, ECG (lead II), and respiratory movements were recorded continuously for 20–30 minutes of control, followed by an infusion of nitroglycerin (32 μg/kg per min, IV) for 15–20 minutes, in order to excite sympathetic activity.[21]

Data Analysis

Off-line analysis was performed on DEC MNC 11/23 and PDP 11/24 minicomputers. ECG, respiration, and pressure data were played back from the FM tape and digitized at 300 samples/sec per channel. The principles of the software for data acquisition and analysis have been described previously.[13,20,22]

Briefly, stationary sections of data both at rest and

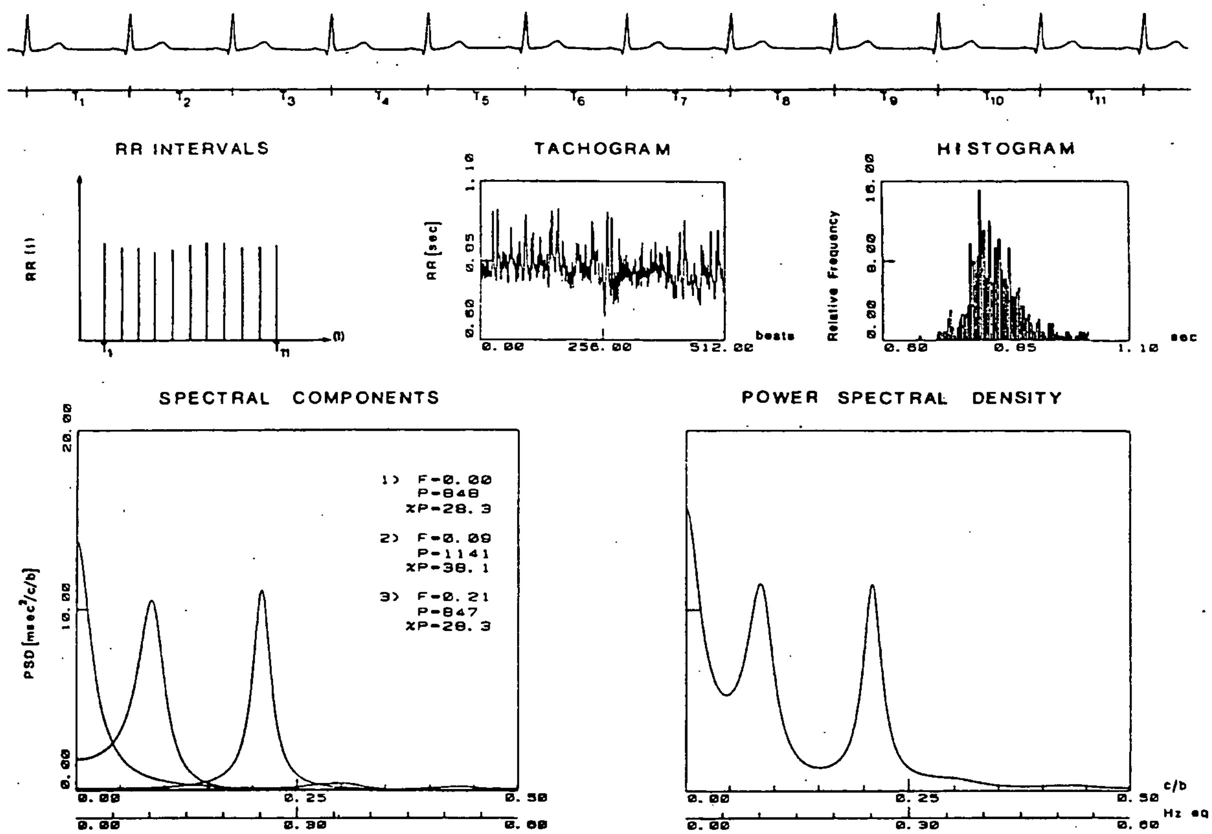

FIGURE 1. *Schematic outline of the computer analysis of R–R variability. From the surface ECG (top trace), the series of R–R intervals is calculated as a function of the beat number. This gives rise to the tachogram and, from it, simple statistics are first computed, such as mean, variance, and the relative frequency distribution, i.e., the histogram. Following this, spectral analysis is performed: Individual spectral components are automatically determined, together with their center frequency and associated power, i.e., area. Finally, the autospectrum is computed and plotted. Inset of left lower panel: F = frequency in cycles/beat; P = power in msec^2; %P = fractional power. In this, as well as in the other R–R interval variability autospectra, the power spectral density (PSD) units should be multiplied by 10^3.*

during tilting of appropriate length were selected for analysis. As schematically represented in Figure 1, the computer program first calculates the interval tachogram, i.e., the series of N consecutive R–R intervals, and saves them in memory. From sections of tachogram of 512 interval values, simple statistics (mean and variance) of the data are computed. This length of the tachogram has been selected as a best compromise between the need for a large time series, in order to achieve greater accuracy in the computation, and the need to obtain stationary recordings, which would be easier for short time periods. As an example, for an average heart rate of about 70/min, 512 successive R–R intervals would amount to approximately 7.5 minutes. The computer program automatically calculates the autoregressive coefficients necessary to define the power spectral density estimate (see the Appendix). An important feature of the program is that it also calculates the model that provides the best statistical estimate and prints out the power and frequency of every spectral component. Each spectral component is presented in absolute units, as well as in normalized form, by dividing it by the total power less the DC component, if present. This component can also be recognized in the graphs of the autospectra on the decaying part of the curve near the origin of the abscissa. Only components greater than 5% of the total power were considered significant. Stationarity was assessed either by pole diagram analysis (see the Appendix) or by verifying that a difference of less than 5% was present between autospectral components calculated in the two successive 256 beat series constituting the whole series of 512 beats.

In this study, the duration of the periodical phenomena in the variability signal was measured as a function of cardiac beats, rather than seconds. As an example, a four-beat periodical component is represented with a frequency of 1:4, i.e., 0.25 cycle/beat. However, this frequency is easily converted into hertz equivalents (Hz Eq) by dividing it by the average R–R interval

length. For instance, if the average R–R length were 1000 msec, this would correspond to 0.25 Hz Eq. In the figures, both units are indicated, whereas, in the text, only hertz equivalents are used.

De Boer et al[23] have compared different methods of spectral analysis of the heart rate variability signal and found that the analysis of the interval series that represents heart period as a function of the beat number not only provides comparable results to methods that represent heart period as a function of time, but is even better suited to the study of the relationship between heart period and arterial pressure on a beat-by-beat basis.

After synchronous acquisition and appropriate calibration, a similar procedure is used to compute the spectrum of the arterial pressure data for both systolic and diastolic values.[20]

Spectral analysis of the impedance respiratory signal was also performed to assess the effects of metronome breathing on respiratory waveform.

Statistics

Data are presented as means ± SE. Student's t test was used to determine the significance of the differences between rest and tilt.

Analysis of variance with Scheffé test was used to assess the effects of age and of β-adrenergic receptor blockade, as well as the effects of controlled breathing.

Differences were considered significant at $p<0.05$.

Regression analysis was used to test the effects of age on R–R interval duration variance.[24]

Results

Age Dependency of Heart Rate Variability

R–R interval duration variance demonstrated a significant age dependency as it decreased with increasing age both at rest and during passive upright tilt (90°) (Figure 2). This effect appeared significantly represented by a curvilinear, i.e., exponential, relationship ($r=0.70$, $p<0.001$ at rest, and $r=0.60$, $p<0.001$ during tilt). Therefore, subjects are subdivided into three age groups of, respectively, 20–30 ($n=30$), 30–45 ($n=10$), and 45–60 ($n=17$) years (Table 1). Furthermore, to account for the possible large differences in total power of individual autospectra, variance data are presented in absolute units, whereas spectral components are normalized by dividing each component by total power (less the DC component, if present).

Table 1 indicates that, at rest, there was a slight tendency for mean R–R interval to increase with age, while R–R variance in the young age group was significantly greater (4097 ± 361 msec²) than both in the mid (2581 ± 356 msec²) or in the old age group (1354 ± 205 msec²).

Effects of Tilt on Heart Rate Variability

Figure 3 depicts a representative example, in a young subject, of the effects of tilting on the time series of R–R intervals, i.e., the tachogram, as well as on the computed autospectrum. The small fluctuations of the instantaneous R–R values around the mean that were present both at rest and during tilt, when analyzed in their nonrandom components, provided two drastically different autospectra. At rest, there were two major spectral components at low (~0.1 Hz Eq) and high (~0.25 Hz Eq) frequency. The normalized area of the low-frequency component (calculated automatically, see "Materials and Methods" and the Appendix) was slightly predominant (58 ± 3%) with an average low frequency:high frequency (LF:HF) ratio of 3.6 ± 0.7 (Table 1). Notice that only about 85% of total variability is represented by the sum of the LF and HF components (Table 1) since in the individual subjects, smaller components could also be present.

During tilt, the majority of R–R variability was represented by a largely predominant LF component (90 ± 1%). However, an HF component was also present (9 ± 1%) although at times very small (Figure 3), with an average LF:HF ratio of 21 ± 4. As shown in Table 1, heart period during tilting underwent a signifi-

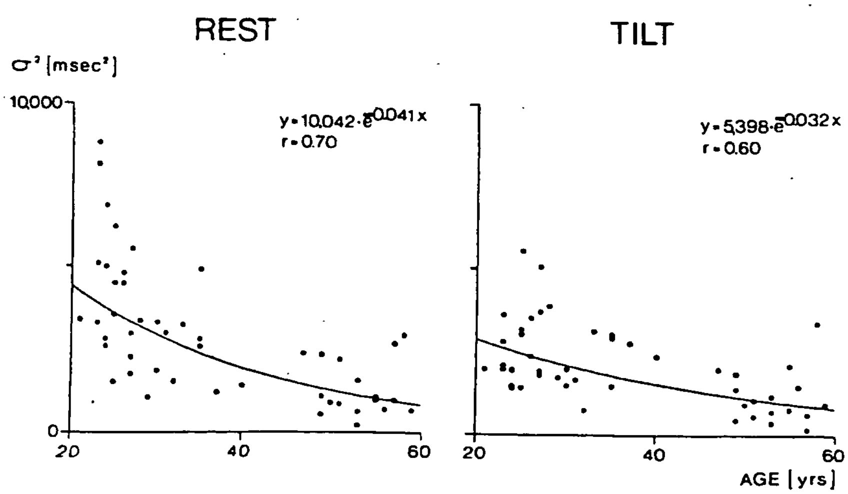

FIGURE 2. *Relationship between R–R interval variability, expressed as variance (σ^2), and age in the study population at rest and during 90° upright passive tilt. Notice that in both cases there is a significant exponential relationship ($p<0.001$, $n=48$).*

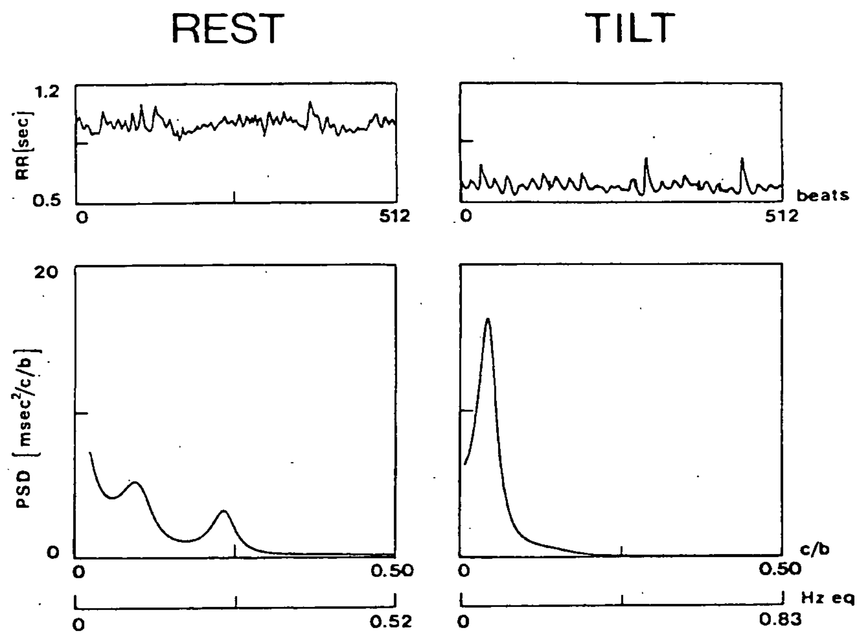

FIGURE 3. *R–R interval series, i.e., tachogram at rest and during passive upright 90° tilt. On the autospectra (bottom panels), two clearly separated low- and high-frequency components are present at rest. During tilt, the low-frequency component becomes preponderant.*

cant reduction, as expected. However, there was no significant correlation between the increases in LF component and in heart rate induced by tilting ($r = 0.19$).

Autospectra of R–R variability in individual subjects remained remarkably constant when repeated over time. As shown in Table 2, there was no difference ($p > 0.05$), at rest and during tilt, in the results obtained in two or three different recording sessions with an interval up to 1 year.

Age Dependency of the Response to Tilt

During tilt in the old age group, mean R–R was significantly longer, and variance smaller, than both in the mid and young age groups (Table 1).

The effects of tilt on the autospectra of R–R interval variability when examined in the three different age groups demonstrated only minor differences, as LF component was smaller and HF component slightly greater in the old age group (Table 1). This difference, however, was no longer apparent in the LF:HF ratio, which was not significantly different in the three age groups both at rest and during tilt (Table 1).

Effects of Controlled Respiration

The effects of controlled respiration were examined in 16 young subjects that were first studied while breathing spontaneously (Figure 4, top panels) and, subsequently, while breathing following a metronome (Figure 4, bottom panels). Respiratory frequency, in

TABLE 2. *Reproducibility of the Effects of Tilt on R-R Variability*

	R-R interval (msec)	R-R variance (msec^2)	Low-frequency component: Normalized power	Low-frequency component: Frequency (Hz Eq)	High-frequency component: Normalized power	High-frequency component: Frequency (Hz Eq)
Rest						
First study (n = 10)	885±43	3264±464	62.0±5.0	0.11±0.01	28.5±3.3	0.26±0.02
Second study (n = 10)	858±32	3508±766	61.4±4.6	0.10±0.01	29.0±3.7	0.25±0.02
Third study (n = 4)	914±34	4201±781	56.1±2.6	0.12±0.01	31.3±3.8	0.31±0.03
Tilt						
First study (n = 10)	672±24*	2612±528	89.5±1.4*	0.09±0.01	6.0±0.5*	0.30±0.04
Second study (n = 10)	676±33*	1955±303	89.2±2.2*	0.10±0.01	6.9±0.07*	0.26±0.03
Third study (n = 4)	700±25*	3381±250	87.0±2.0*	0.10±0.01	7.7±1.3*	0.27±0.02

Average delay in time between the first and second study: 138 days (range 7–365). Average delay in time between the second and third study: 127 days (range 90–150).
*Value during tilt significantly different from value at rest ($p < 0.05$).

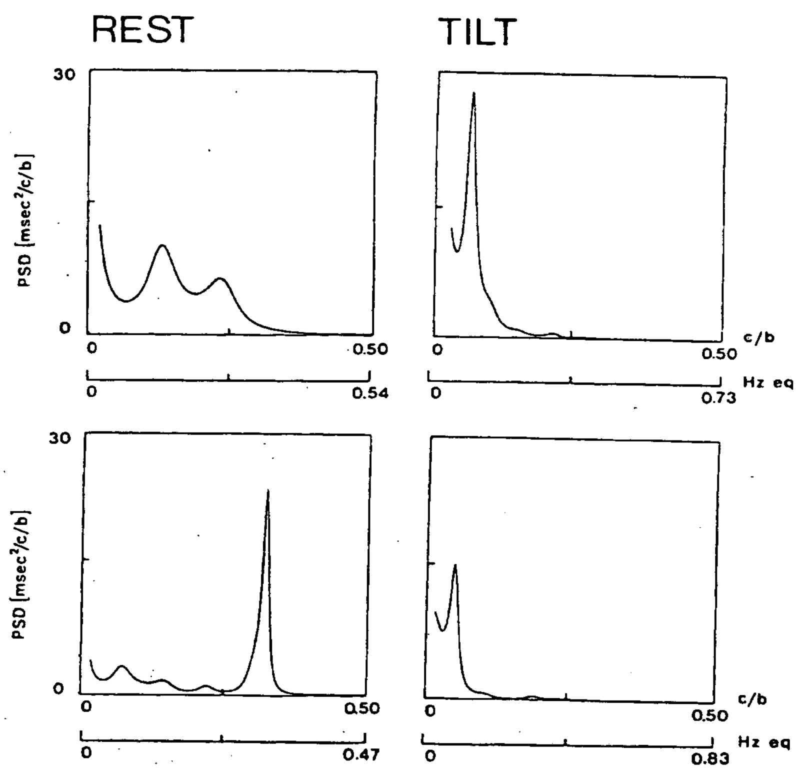

FIGURE 4. *Representative example of autospectra of R–R interval variability during spontaneous breathing (top panels) and during controlled respiration, at 20/min (bottom panels). Note that, with controlled respiration at rest, the relative power of the high-frequency component becomes predominant.*

the latter condition, was set at 20/min, i.e., 0.33 Hz, in order to separate better the low-frequency from the high-frequency respiratory component.

This maneuver modified the respiratory waveform, as demonstrated by spectral analysis: during spontaneous respiration, nonstationarities in the signal are reflected by a diffuse profile of the spectrum, whereas during metronome breathing, the near sinusoidal shape of the respiratory waveform is reflected by a single narrow component in the spectrum. In either condition, the HF component of the spectrum of R–R variability coincided with the main respiratory frequency. The LF component never coincided with a major component of the spectrum of the respiratory waveform.

During controlled respiration, at rest, there was a significant reduction of the LF component with a concomitant increase in the HF component (Figure 4, bottom; Table 3). The LF:HF ratio was significantly reduced from 2.5 ± 0.3 to 0.7 ± 0.1. As the subjects were allowed to adjust their tidal volume, during spontaneous respiration the tidal volume ($n = 7$) was 552 ± 98 ml, and slightly less (533 ± 76 ml) during controlled breathing at 20/min. During metronome breathing, there was only a small but not significant change in transcutaneous P_{CO_2} and P_{O_2} (respectively, 42 ± 2, 83 ± 4 mm Hg during spontaneous and 38 ± 2, 79 ± 6 mm Hg during controlled breathing, $p > 0.05$). During tilt, the HF component was de-

TABLE 3. *Effects of Metronome Breathing on R-R Variability (n = 16)*

	R-R interval (msec)	R-R variance (msec²)	Low-frequency component: Normalized power	Low-frequency component: Frequency (Hz Eq)	High-frequency component: Normalized power	High-frequency component: Frequency (Hz Eq)
Rest						
Free resp frequency Hz 0.26 ± 0.02	884 ± 27	4095 ± 436	56.2 ± 3.9	0.11 ± 0.01	27.3 ± 2.7	0.24 ± 0.02
			*		*	*
Control resp frequency Hz 0.33	934 ± 29	4220 ± 541	28.9 ± 4.3	0.11 ± 0.01	51.6 ± 4.4	0.33 ± 0.01
Tilt						
Free resp frequency Hz 0.26 ± 0.03	678 ± 21†	2647 ± 308†	86.4 ± 2.9†	0.10 ± 0.01	7.8 ± 1.4†	0.25 ± 0.02
			*		*	*
Control resp frequency Hz 0.33	705 ± 22†	2119 ± 428†	71.6 ± 5.3†	0.10 ± 0.01	15.3 ± 3.0†	0.31 ± 0.02

*Significant difference ($p < 0.05$).
†Value during tilt significantly different from value at rest ($p < 0.05$).

creased while the LF component was increased, as expected; however, this LF increase was less pronounced than during spontaneous breathing (Figure 4). Consequently, the increase in LF:HF ratio induced by tilt was smaller during controlled respiration (8.3 ± 1.6) than during spontaneous breathing (16.0 ± 3.0).

Furthermore, during tilt, tidal volume was slightly smaller with controlled respiration than with spontaneous breathing (574 ± 100 and 657 ± 48 ml, respectively), and no significant change ($p > 0.05$) was observed in transcutaneous P_{CO_2} or P_{O_2} (respectively, 39 ± 4, 87 ± 5 mm Hg with spontaneous and 43 ± 3, 82 ± 4 mm Hg with metronome breathing).

β-Adrenergic Receptor Blockade

The effects of β-adrenergic receptor blockade were assessed in two sets of experiments. Acute β-adrenergic receptor blockade was obtained in 10 young subjects by IV bolus of 0.2 mg/kg of propranolol that reduced significantly heart rate and arterial blood pressure (Table 1). Under these conditions, R–R interval variance was significantly increased from control (Figure 5; Table 1), but normalized autospectral components were not modified significantly (Table 1). During tilt, there was a significantly lesser reduction in R–R interval and a smaller increase in the LF component and in the LF:HF ratio (Figure 5; Table 1).

Chronic β-adrenergic receptor blockade was obtained in 12 young subjects with 0.6 mg/kg p.o. propranolol t.i.d. for 6 days, which reduced significantly resting heart rate and arterial blood pressure. The effects on resting R–R interval variability, like acute blockade, were characterized by augmented variance (Figure 5; Table 1). However, marked changes were observed in the resting autospectra: LF was smaller, HF greater, and hence, LF:HF significantly reduced, compared with controls (Table 1; Figure 5). During tilt, the LF component was reduced and HF component increased, compared with control. Thus, during chronic β-adrenergic receptor blockade, LF:HF ratio increased to only 3.78 ± 0.93, which was smaller than that observed in the young age group without β-blockade during tilt (20.79 ± 3.68) but was similar to their resting value (3.62 ± 0.70).

Simultaneous R–R and Blood Pressure Variabilities

In a group of 7 normotensive subjects (20–60 years old) in whom systemic arterial pressure was recorded with direct high-fidelity techniques (see "Materials

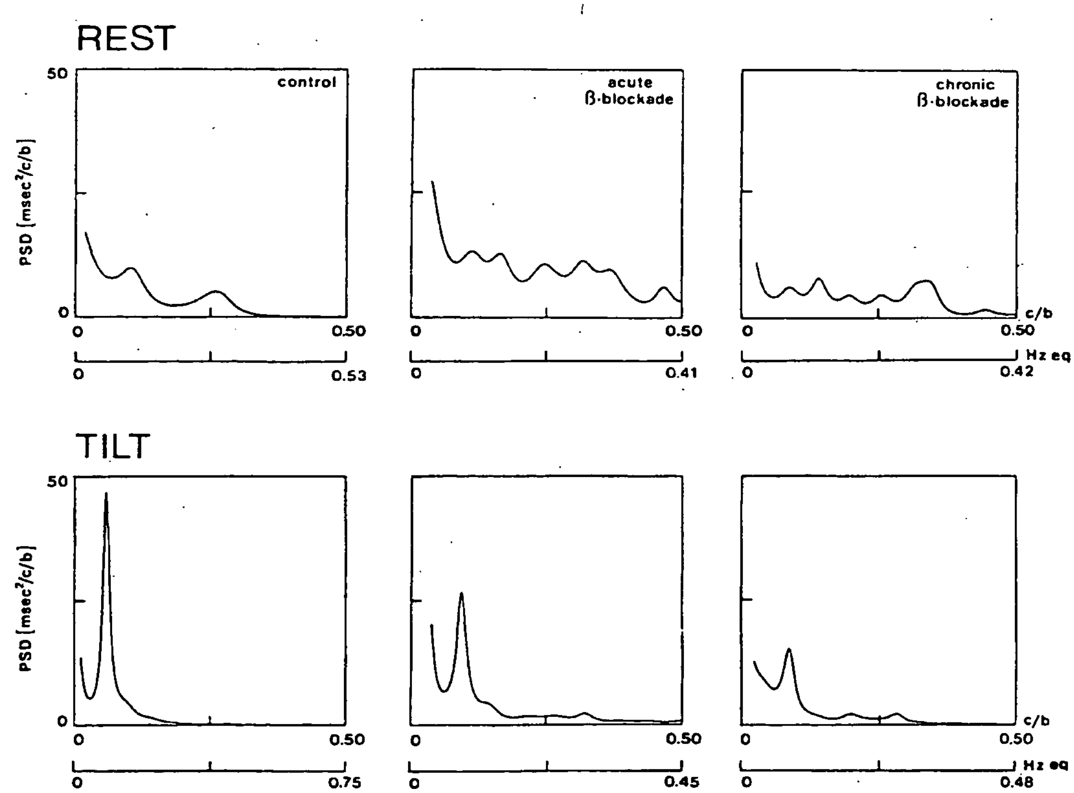

FIGURE 5. *Autospectra of R–R interval variability of a young subject at rest (top panels) and during tilt (bottom panels) under control conditions (left panels) and during acute (0.2 mg/kg IV propranolol, middle panels), and chronic (0.6 mg/kg p.o. propranolol t.i.d.) β-adrenergic receptor blockade. Note the progressive reduction in the relative power of the low-frequency component during tilt.*

and Methods") simultaneously with the ECG, the analysis of the variability of systolic and diastolic beat-by-beat values was also performed.

Systolic arterial pressure variability was characterized by small oscillations in the time series of beat-by-beat values (Figure 6), resulting in a tracing comparable to the interval tachogram.

Moreover, the autospectra of systolic arterial pressure variability demonstrated two major components, respectively, a LF of 41 ± 8% and HF of 19 ± 5%, with a relative ratio of 2.8 ± 0.7 at rest. During tilt, the LF component became largely predominant (73 ± 8%), with an increase in the LF:HF ratio of 17 ± 5. Spectral analysis of diastolic blood pressure gave similar results (Table 4). It should be appreciated that the simultaneous autospectra of R–R variability (Figure 6; Table 4) provided results resembling those obtained from the analysis of arterial pressure variability, with two components recognized at the same frequencies, with similar relative ratios.

As shown in Figure 7, cross-spectral analysis of R–R and blood pressure variability confirmed this evaluation by showing coherence only between the same LF and HF components both at rest and during tilt. The phase difference between R–R and arterial pressure variability was approximately 0° at HF. At LF, the phase presented different patterns in rest and in tilt conditions: In the former case, the phase had a linear relation with frequency, whereas, in the latter case, it oscillated around a fixed value. In both cases, the phase corresponding to the central frequency of the LF peak was about 60°, i.e., ⅙ of the entire 360° cycle. As LF corresponds to a period of about 10 beats, the calculated phase difference amounted to ⅙ of that period, i.e., to a delay of 1.7 beats, with pressure leading.

R–R and Arterial Pressure Variability in Conscious Dogs

To assess more directly the effects of sympathetic innervation on heart rate variability, we performed experiments on a group of conscious dogs before and after bilateral stellectomy.

As shown in Figure 8 and Table 5, under control conditions R–R variability was almost solely represented by a HF respiratory-linked component, whereas a very small (8 ± 2%) LF component was present in only 50% of the animals. Similarly (Table 5), the autospectra of both systolic and diastolic blood pressure variabilities demonstrated a major HF component. During moderate hypotension (−9 ± 2% from 85 ± 3 mm Hg mean arterial pressure) obtained by a continuous infusion of IV nitroglycerin (32 μg/kg per minute), heart rate increased 47 ± 9% from 82 ± 5 beats/min as a consequence of sympathetic activation.

Under those conditions, not only was there a significant reduction of total power of R–R variability, but also the HF component of the autospectrum was reduced to 42 ± 3%, whereas, in all animals, a LF component of similar power (36 ± 6%) became evident. Similar changes were observed in arterial pressure autospectra (Table 5). Bilateral stellectomy did not significantly modify arterial pressure, heart rate, or R–R or pressure variability, as expressed both by variance or by their autospectra (Figure 8; Table 5). The IV infusion of nitroglycerin, repeated at the same dose, reduced arterial pressure and increased heart rate to a similar extent (Table 5). The response of R–R variability, however, was modified. Although total power was reduced to a value similar to that observed in the intact animals, only a HF component could be observed in the autospectrum. Conversely, the increase in LF components of pressure autospectra was essentially preserved (Table 5).

Discussion

This study in man examines the beat-to-beat oscillations which characterize heart rate and arterial pressure under various steady state conditions, in the hypothesis that the quantitative information provided by the spectral analysis of these oscillations reflects the interaction between sympathetic and parasympathetic regulatory activities.

Various approaches have been proposed to evaluate the contributions of parasympathetic and sympathetic discharges[25] to the heart rate variability. Parasympathetic activity has been clinically inferred from peak-to-peak variations of the heart period either in the clinical laboratory environment[26] or with Holter moni-

TABLE 4. *Effects of Tilt on Simultaneous R-R Interval and Arterial Pressure Variabilities (n = 7)*

	Mean value	Variance	Low-frequency component: Normalized power	Low-frequency component: Frequency (Hz Eq)	High-frequency component: Normalized power	High-frequency component: Frequency (Hz Eq)
Rest						
R-R	748 ± 20 msec	1403 ± 571 msec²	41.2 ± 8.7	0.12 ± 0.01	19.4 ± 5.3	0.33 ± 0.03
SAP	125 ± 5 mm Hg	20 ± 3 mm Hg²	46.7 ± 7.2	0.11 ± 0.02	27.5 ± 9.0	0.32 ± 0.03
DAP	70 ± 5 mm Hg	15 ± 3 mm Hg²	48.5 ± 11.7	0.10 ± 0.01	31.1 ± 7.7	0.35 ± 0.04
Tilt						
R-R	633 ± 29* msec	1483 ± 643 msec²	73.4 ± 8.6*	0.09 ± 0.01	7.4 ± 1.9*	0.33 ± 0.02
SAP	129 ± 6 mm Hg	47 ± 10* mm Hg²	78.7 ± 4.1*	0.09 ± 0.01	9.2 ± 2.1	0.32 ± 0.03
DAP	74 ± 4 mm Hg	29 ± 7* mm Hg²	73.3 ± 2.5*	0.08 ± 0.01	9.9 ± 1.7*	0.35 ± 0.03

SAP = systolic arterial pressure; DAP = diastolic arterial pressure.
*Value during tilt significantly different from value at rest ($p < 0.05$).

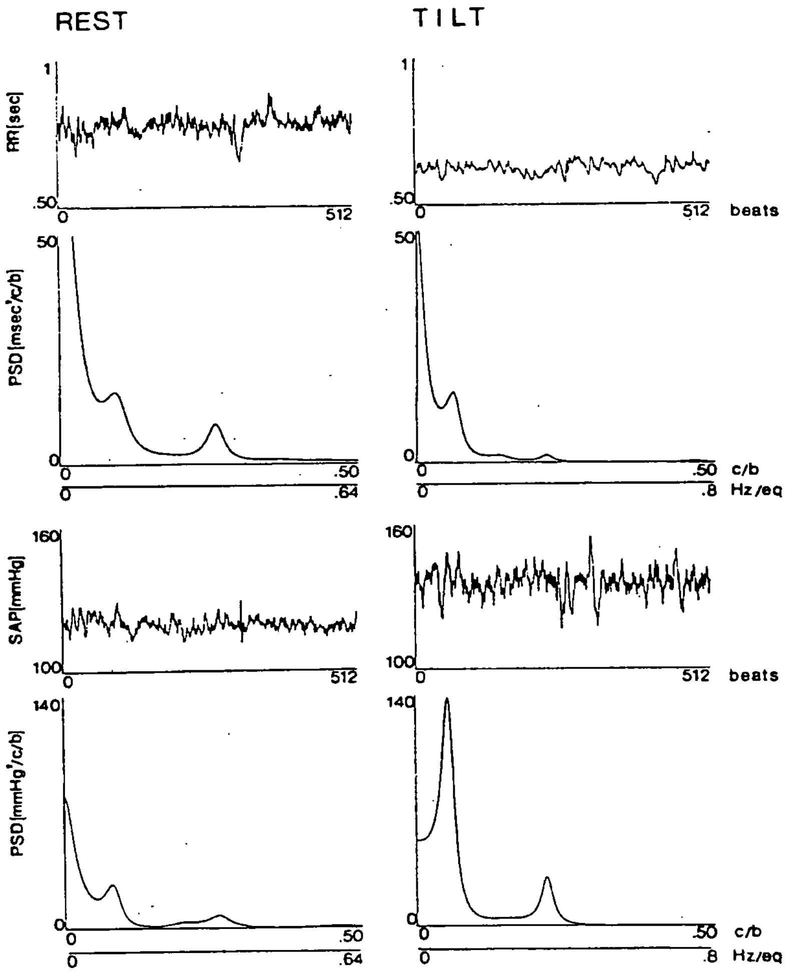

FIGURE 6. *Simultaneous analysis of R–R interval and systolic arterial pressure variabilities in a young subject. Note the reduction in average R–R interval and the slight increase in average systolic arterial pressure on passing from rest to tilt. The two components of the autospectrum of systolic arterial pressure variability are similar to those of R–R interval variability at rest and during tilt.*

toring.[27] A different approach has been used by others[10,12,15] who utilized spectral techniques to assess the frequency components of the heart rate variability signal. A possible major advantage of spectral analysis is the observation that changes in the sympathetic activity to the heart can also be recognized, and hence, some index of the instantaneous balance between sympathetic and vagal activity can be obtained.[15] It should be noted that, since heart rate variability signal is a pseudorandom phenomenon, previous studies using the fast Fourier transform to compute the power spectrum have some technical limitations (see the Appendix). They include the deterministic nature of the algorithms used, which, in principle, are applicable only to periodical phenomena,[16] the need of windowing the data, and the difficulty in defining with certainty the relative power of the various spectral components.

In the present study, some of these limitations were avoided because the autoregressive algorithms, which are applicable to nonperiodical phenomena,[28] computed automatically the number and relative power of the various spectral components. Moreover, there was no need for windowing or filtering the data.[13,22]

Spectral Analysis of Heart Rate Variability and Parasympathetic Activity

In our analysis, we observed at rest two consistent major spectral components, a LF at ~0.1 Hz and an HF at ~0.25 Hz. The LF component seems to correspond to the Mayer waves,[5,17] while the HF component is synchronous with the respiration and has been considered as a quantitative evaluation of respiratory arrhythmia.[15] Since the HF component disappears after atropine, it could represent a clinically useful index of vagal activity.[15]

In our study, during spontaneous quiet breathing at rest, a relatively small area was found to be associated with the HF component. However, Pomeranz et al[15] found a predominant HF component in 8 subjects who breathed following a metronome at 15/min. A similarly enhanced HF component was found in this study in 16 subjects who breathed following a metronome at

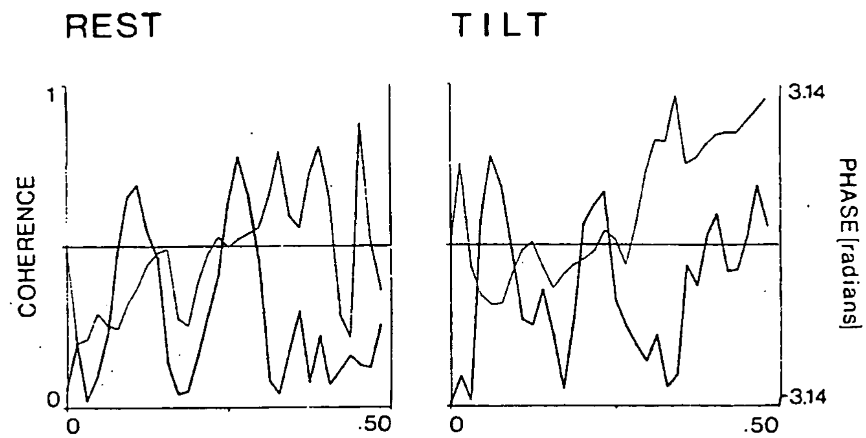

FIGURE 7. *Cross-spectral analysis (coherence, thick lines; phase, thin lines) between R–R interval and systolic arterial pressure variabilities computed from the data of Table 4. Note that, both at rest and during tilt, only two spectral components show a coherence greater than 0.5 in both conditions. Phase is about 0° at the respiratory component and negative, i.e., pressure leads R–R interval, at the 0.1-Hz component.*

20/min. The same subjects showed a predominant LF component when breathing spontaneously. Changes in the amount of respiratory arrhythmia and, hence, of the HF component could reflect changes in volume, as well as in frequency of respiration.[29,30] Important modulatory mechanisms could also be initiated by changes in arterial P_{O_2} and P_{CO_2}, and thereby changes in neural efferent activities,[31] as well as by variations in arterial pressure and consequent alterations in baroreceptor input.[32]

In this study, we could not define the exact mechanisms responsible for the observed increase in HF component with controlled breathing, as tidal volume, transcutaneous P_{O_2} and P_{CO_2}, and arterial pressure did not change significantly.

However, an important role is likely to be played by the marked change in respiratory waveform,[33] acting either through a more efficient stimulation of lung receptors[34] or through fluctuations induced in arterial pressure.[32,35] In either case, the increased HF component of R–R variability would indicate that during metronome breathing there is enhanced vagal tone.

TABLE 5. *Effects of Moderate Hypotension on Simultaneous R-R Interval and Arterial Pressure Variabilities in Conscious Dogs*

			Low-frequency component		High-frequency component	
	Mean value	Variance	Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)
Part A						
Intact (n = 8) control						
R-R	755 ± 62 msec	58237 ± 17754 msec^2	4 ± 2	0.14 ± 0.01	63 ± 6	0.24 ± 0.02
SAP	119 ± 18 mm Hg	217 ± 112 mm Hg^2	19 ± 4	0.12 ± 0.02	60 ± 4	0.23 ± 0.03
DAP	71 ± 3 mm Hg	161 ± 71 mm Hg^2	15 ± 5	0.10 ± 0.03	64 ± 4	0.24 ± 0.03
Nitroglycerin (32 μg/kg per min)						
R-R	521 ± 37* msec	7513 ± 3941* msec^2	36 ± 6*	0.12 ± 0.01	42 ± 4*	0.26 ± 0.03
SAP	104 ± 5* mm Hg	45 ± 19 mm Hg^2	55 ± 4*	0.09 ± 0.01	27 ± 5*	0.27 ± 0.06
DAP	66 ± 2 mm Hg	50 ± 21 mm Hg^2	59 ± 7*	0.10 ± 0.01	22 ± 5*	0.25 ± 0.03
Part B						
Stellectomy (n = 5) control						
R-R	790 ± 41 msec	42786 ± 14370 msec^2	3 ± 2	0.10 ± 0.01	61 ± 5	0.23 ± 0.01
SAP	137 ± 10 mm Hg	67 ± 28 mm Hg^2	8 ± 3	0.11 ± 0.01	72 ± 4	0.25 ± 0.01
DAP	76 ± 3 mm Hg	78 ± 22 mm Hg^2	8 ± 4	0.09 ± 0.01	63 ± 7	0.23 ± 0.02
Nitroglycerin (32 μg/kg per min)						
R-R	617 ± 19* msec	3491 ± 1696* msec^2	0 ± 0†	Unmeasurable	76 ± 8†	0.24 ± 0.02
SAP	110 ± 7* mm Hg	16 ± 4 mm Hg^2	38 ± 8*	0.08 ± 0.01	46 ± 10*	0.26 ± 0.02
DAP	73 ± 4 mm Hg	24 ± 13 mm Hg	44 ± 6*	0.09 ± 0.01	37 ± 7*	0.25 ± 0.02

SAP = systolic arterial pressure; DAP = diastolic arterial pressure.
*Value during nitroglycerin infusion significantly different from control ($p < 0.05$).
†Significant difference ($p < 0.05$) between value in intact and denervated animals.

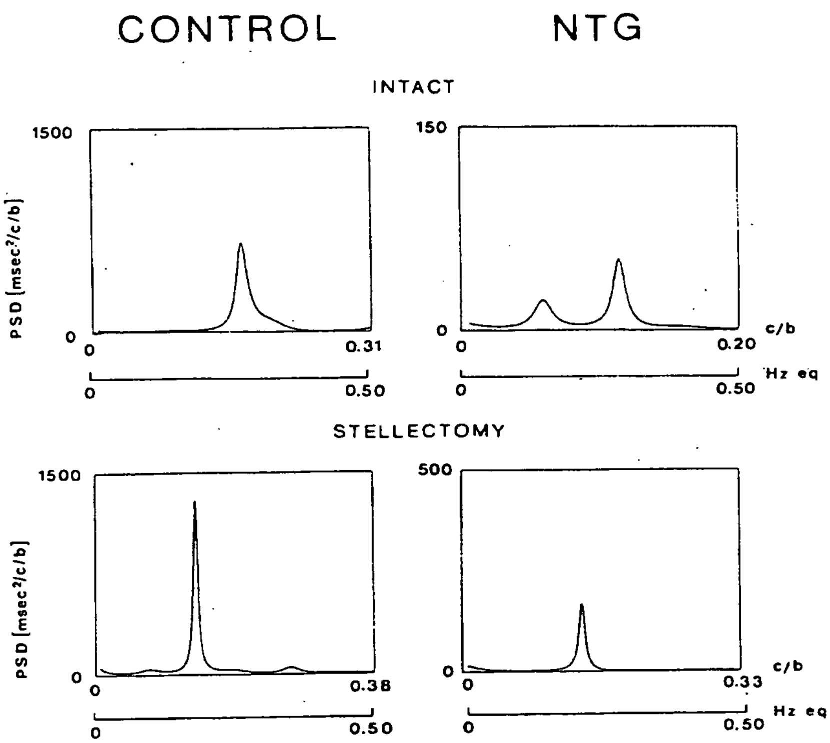

FIGURE 8. *Autospectra of R–R interval variability of a conscious healthy dog in control conditions (left panels) and during an IV infusion of nitroglycerin to excite the sympathetic outflow. The top panels were obtained with cardiac nerves intact, the bottom panels after full recovery from bilateral stellectomy. Note the presence of a large low-frequency component during nitroglycerin infusion only in the neurally intact situation.*

Spectral Analysis of Heart Rate Variability and Sympathetic Activity

The possibility that spectral analysis could provide an index of sympathetic activity is less well established. In animal studies, sympathetic activity has been considered either to play no role[10] or else to be instrumental in the genesis of the LF rhythm.[12,36] Furthermore, electroneurographic recordings in dogs[25] suggest that sympathetic activity might also modulate respiratory arrhythmias. This apparent discrepancy of results is probably a consequence of the differences in the preparations used, i.e., anesthetized and acutely decerebrated cats[10] as opposed to conscious dogs.[12]

In our study, we tested the hypothesis that spectral analysis of heart rate variability could provide an assessment of sympathetic tone, by planning experiments in man in which sympathetic activity was either increased functionally or blocked pharmacologically. Enhanced sympathetic drive to the heart, as obtained by an orthostatic stimulus, was constantly associated with a marked increase in the LF and with a decrease in the HF component of the autospectrum. We selected a passive change of posture in order to minimize the influence on heart rate exerted by the muscular effort of active standing.[37]

Acute β-adrenergic receptor blockade with intravenous propranolol, besides reducing heart rate, had a marked effect on R–R variability. At rest variance increased, while the relative contribution of LF and HF components remained relatively unchanged. During tilt, there was a significant reduction of the increase of the LF component and of the LF:HF ratio. Similar blunting effects exerted by acute β-adrenergic receptor blockade were observed during active standing by Pomeranz et al.[15]

After chronic β-adrenergic receptor blockade, there was, at rest, not only an increase in R–R interval variance similar to that observed with acute blockade, but also a significant effect on spectral components. Thus, LF and LF:HF were significantly smaller, and HF was significantly greater than under control conditions. During tilt, the increase in LF component and in the LF:HF ratio was markedly reduced; indeed, the numerical value of the ratio was similar to that observed under resting control conditions without β-blockade.

It might be pertinent to recall that Wallin et al[38] demonstrated, with electroneurographic techniques in hypertensive subjects, that chronic β-adrenergic blockade was effective in reducing sympathetic efferent activity, whereas acute blockade was ineffective.

As to the effects of metronome breathing on LF, this component was reduced at rest, and its increase during tilt was blunted, possibly as a consequence of the inhibitory effect on sympathetic rhythms produced by pulmonary afferent activity.[34]

As to the effects of aging, it was progressively associated with a reduced R–R variability as expressed by variance, and during tilt with a smaller activation of LF and a smaller reduction of HF. However, the changes in LF:HF ratio during tilt were not significantly different among the various age groups. This approach

would suggest that aging is characterized by a new equilibrium between the two sections of the visceral nervous system rather than by alterations limited to the sympathetic outflow.[39,40]

Simultaneous Variability of R–R Interval and Arterial Pressure

An additional major observation of this study is that the autospectra of R–R interval and of both systolic and diastolic arterial pressure beat-to-beat variabilities were similar at rest and showed parallel changes with tilt. The presence in arterial pressure recordings of an HF respiratory component has been traditionally interpreted as a mechanical consquence of respiration, which could act directly on intrathoracic vessels or indirectly through changes in stroke volume[7,41] and heart period.[36] Sympathetic modulation of arterial smooth muscle is probably too slow to follow the 0.25-Hz respiratory frequency.[42] Obviously, these beat-to-beat pressure changes could affect R–R interval through complex reflex adjustments,[43] among which baroreflexes could have a paramount importance.[44]

The LF components correspond to the well-known Mayer[5] waves, a phenomenon that, although described in quite artificial experimental conditions, seems to pertain to normal human subjects[8] as well. Various theories, including myogenic oscillations, central rhythms, feedback mechanisms, and "resonance" disturbances, have been advanced for their interpretation.[17]

As to the effects of tilt, arterial pressure beat-by-beat variability, as expressed by variance, increased significantly for both systolic and diastolic values. Furthermore, the LF oscillatory components increased signficantly and to an extent similar to that observed in the autospectrum of R–R interval, whereas HF components were, in both autospectra, markedly reduced.

In this respect, it should be mentioned that an increase in arterial pressure variability has been described also in essential hypertension,[45] and a greater LF component has been documented in daytime as opposed to nighttime recordings in ambulatory patients.[46] Both conditions are possibly characterized by a higher sympathetic activity.

Cross-spectral analysis of systolic arterial pressure and R–R interval variabilities indicated that a high degree of coherence existed between the fluctuations of these two variables both in recumbency and during tilt. In correspondence to the HF component, arterial pressure and R–R interval changes occurred in phase, whereas each LF pressure change preceded R–R interval oscillation by about two beats.

Although this cross-spectral analysis provides no direct insight into the mechanisms linking heart period and arterial pressure oscillations, with their possible neural and non-neural components,[12,47] it supports the conclusion that similar information on oscillatory rhythms can be obtained from both invasive and noninvasive studies, not only at rest, but also during augmented sympathetic activity.

In a previous study by De Boer et al,[47] only a small HF component was observed in the autospectrum of diastolic arterial pressure measured in sitting subjects either with direct or indirect plethysmographic clamp method.[48] Technical differences in arterial pressure recording and the intermediate level of gravitational stimulation induced by sitting should account for the difference in our data.

Conscious Dogs

More direct information on the role of cardiac sympathetic nerves was derived from a group of experiments in conscious dogs before and after bilateral stellectomy. In the conscious dog, the LF components are, under control conditions, either absent[33] or variable[12,36] and small (Table 5). An excitation of sympathetic activity as induced by moderate hypotension obtained with phentolamine[36] or, as in our experiments, with nitroglycerin, was associated with an increase in LF components. This increase was no longer present after bilateral stellectomy, which, on the other hand, did not modify the LF and HF components characterizing the arterial pressure variability (Table 5). Hence, in conscious dogs that had fully recovered from surgery, it was possible for the first time to dissociate the LF components present in the heart rate and arterial pressure autospectra by selectively interrupting the cardiac sympathetic loop.

Changes in LF:HF Ratio as Markers of Changes in Sympatho-Vagal Balance

It has been a rather simple but traditional working hypothesis that conditions which increase sympathetic activity decrease vagal tone and vice versa.[48] However, in electrophysiological experiments, recordings of the sympathetic and vagal discharges, directed to the heart, indicated both a reciprocal[50] and a nonreciprocal[31] organization.

The actual balance between these two outflows is likely to have paramount importance not only under such physiological conditions as gravitational stimuli, exercise, and changes in central command,[51] but also during disturbances such as arrhythmias,[52] myocardial ischemia, arterial hypertension,[53] or alterations in the renin-angiotensin system.[12]

It has recently been suggested that spectral analysis of R–R interval variability might reflect this balance.[15] In our study, the LF:HF ratio appeared to be a convenient index of such interaction. Indeed, the simultaneous increase in vagal and decrease in sympathetic mechanisms produced by metronome breathing were reflected by a reduction of LF:HF ratio at rest and by a blunting of the increase induced by tilt. Furthermore, LF:HF ratio appeared to follow the reductions of sympathetic activity produced by acute and chronic β-adrenergic receptor blockade both at rest and during tilt. Clinical research into those states characterized by a disturbance in the neural regulatory mechanisms will probably reveal in the near future whether this approach will have true pathophysiological significance.

Appendix

The basic assumption underlying the proposed signal processing methods is that heart rate and systolic and diastolic

blood pressure values fluctuate cycle by cycle, even in stable conditions, around a given mean value. Beat-to-beat heart rate and arterial blood pressure variability signals are obtained first from the original biological signals as a discrete time series y(k) where k is the progressive number of detected events.

Variability signals are intrinsically pseudo-random and can be considered as the realization of a stochastic process y(k) which is the output of a linear time-invariant system driven by white noise w(k), which constitutes the random component of the model and is fully characterized by a mean value which is zero for simplicity of notation and a variance λ^2 [i.e., $w(k) = WN(O,\lambda^2)$] according to the model

$$w(k) \rightarrow \boxed{H(z)} \rightarrow y(k),$$

where H(z) is the transfer function in the complex variable z, which is determined in a quantitative way and is the model of the signal-generating mechanism. A simple structure of input/output relation is given by the autoregressive (AR) modelization

$$y(k) = \sum_{i=1}^{p} a(i)y(k-i) + w(k)$$

where a(i) are the p unknown parameters of indentification. More complex models are described by Box and Jenkins.[28] Obviously,

$$H(z) = \left(1 - \sum_{i=1}^{p} a(i)\cdot z^{-i}\right)^{-1}$$

which relates the transfer function with the identification coefficients. Hence, given N samples of variability signals, the problem is to estimate H(z) or, more precisely, the vector θ of parameters

$$\theta = |\, a(1), a(2), a(3) \, . \, . \, . \, . \, . \, a(p), \lambda^2 |$$

The algorithm applied for such an identification is the Batch least squares method via Levinson-Durbin recursion.[18]

Two tests are used to check the validity of the assumed model. The first one is Anderson's test, which measures the whiteness of the prediction error: The identification is not accepted if the test is not satisfied within 5% confidence interval. After fulfilling Anderson's test, the order of the model is chosen as the one which minimizes Akaike's final prediction error (FPE) figure of merit.[54] In this way, the model is completely determined by order p and vector θ.

Power spectral density (PSD) estimation P(f) is obtained from the following relation

$$P(f) = \lambda^2 / \left| 1 - \sum_{i=1}^{p} a(i)\exp(-j2\pi fi) \right|^2$$

where f is the frequency and the sampling period is unitary. The resulting PSD satisfies the criterion of maximum entropy[16] and presents many advantages in respect to the methods based on classical Fourier analysis (FFT algorithms),[18] namely, a more consistent and smoother spectral estimation, a spectral resolution which is independent of the number N of samples, and the possibility of avoiding windowing procedures.

Another important advantage of AR modeling with respect to the more traditional techniques is the possibility of decomposing the autocorrelation function and, hence, its transform in the frequency domain (i.e., the power spectrum density) in single components by applying the residuals theorem.[55] In this way, the AR spectrum also provides the individual spectral components in terms of center frequency and of the corresponding power in absolute, fractional (see Figure 1), and normalized values. The spectrum is calculated on 512 consecutive cardiac beats; stationarity has been tested in two ways: (1) Calculation of mean values and variance of variability signals on consecutive records, by verifying that the values are inside a confidence level of 5%, (2) construction of the pole diagram of the autoregressive model, together with the confidence interval in the pole estimation, by verifying that consecutive records are characterized by poles which are inside the fitted confidence interval (wide-sense stationarity). The spectra which are presented in this paper satisfy both of the preceding conditions.

With the AR spectral estimation presented in this paper, it is possible to improve the statistical consistency, even of very low frequency components (less than 0.02 Hz Eq) which correspond to oscillations in the signal having a period comparable or superior to the considered number of cardiac beats (512). These rhythms are always present in the signals and may have an important physiological meaning, the analysis of which is outside the aim of the present paper. The method of variability signal processing presented has the advantage of decreasing the estimation variance of their power.

The multivariate analysis (cross-spectra and phase spectra) between R–R interval and systemic arterial pressure variabilities are obtained through the calculation of the complex cross-spectrum $P_{xy}(f)$ where x(k) and y(k) are two synchronous discrete time series.

$$P_{xy}(f) = X(f)\cdot Y^*(f)/N = G_{xy}(f)\exp[j\phi_{xy}(f)]$$

where X(f) and Y(f) are the discrete Fourier transforms of the series (* denotes the complex conjugate), G_{xy} is the amplitude cross-spectrum, and ϕ_{xy} is the phase spectrum. The squared coherence k^2 is then obtained as

$$k^2_{xy}(f) = G_{xy}(f)^2/[P_x(f)\cdot P_y(f)].$$

The cross-spectrum is calculated via FFT algorithm, using a triangular window (Bartlett method)[56] on successive overlapping records of 64 samples each. Results are averaged over the whole set of 512 samples. The applied segmentation is a compromise between frequency resolution and estimated consistency.[57]

Squared coherence has values between 0 and 1 and should be considered as analogue to the squared correlation coefficient r^2 in linear regression analysis. Values over 0.5 indicate a significant phase link between R–R interval and pressure variability signals. Thus, only spectral components with high coherence demonstrate a stable phase-shift between instantaneous R–R interval and arterial blood pressure. Its value can be evaluated as a function of frequency.

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KEY WORDS • spectral analysis • variability signals • heart rate • high-fidelity arterial pressure • sympatho-vagal interaction

or HR. The HR power spectrum, in particular, indicated a striking autonomic imbalance immediately after the induction of anesthesia despite stable HR and BP. LFA and LFA/RFA ratio appeared to track sympathetic autonomic activation during abdominal surgical stimulation, but not during other perioperative stressor events.

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Title

Emotions and respiratory patterns: Review and critical analysis.

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Abstract

The literature on emotions and respiration is reviewed. After the early years of experimental psychology, attention to their relationship has been sparse, presumably due to difficulties in adequate measurement of respiration. The available data suggest nevertheless that respiration patterns reflect the general dimensions of emotional response that are linked to response requirements of the emotional situations. It is suggested that the major dimensions are those of calm-excitement, relaxation-tenseness, and active versus passive coping. Research on the emotion-respiration relationships has been largely restricted to the correlates of respiration rate, amplitude, and volume. Finer distinctions than those indicated may well be possible if a wider range of parameters, such as the form of the respiratory cycle, is included in the investigation.

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8/7/2
DIALOG(R)File 352:Derwent WPI

011737085
WPI Acc No: 1998-153995/199814
Oral hypnotic agent used for medicines, foods, drinks and feeds - contains essential oil of sandalwood
Patent Assignee: KOBAYASHI SEIYAKU KK (KOBA)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 10025245	A	19980127	JP 96182349	A	19960711	199814	B

Priority Applications (No Type Date): JP 96182349 A 19960711
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 10025245	A		8	A61K-035/78	

Abstract (Basic): JP 10025245 A

Oral hypnotic agent used for medicines, foods and drinks and feeds contains an essential oil of sandalwood.

The hypnotic medicine preferably contains 0.1-640 mg/kg essential oil. The hypnotic foods and drinks contain 0.005-20 wt.% essential oil, particularly cookies, biscuits, jellies, marshmallows, soft drinks and cocoa. Essential oil of sandalwood is added to medicines, foods, drinks and feeds in an amount of 0.0001-100 w/w%. The agent is administered at doses of 0.1-640 (preferably 45-160) mg/kg.

ADVANTAGE - Good sleep is obtained without side effects.

Dwg.0/1

Derwent Class: B04; D13; D23
International Patent Class (Main): A61K-035/78
International Patent Class (Additional): A23G-001/00; A23G-003/00; A23K-001/16; A23L-001/30; A23L-001/307; A23L-002/00; A61K-009/48; C11B-009/00

8/7/1
DIALOG(R)File 352:Derwent WPI

012930449
WPI Acc No: 2000-102296/200009
Cosmetics showing sedative and hypnotic effect - comprising flavour of rosemary, lavender, bitter orange or Senkyu
Patent Assignee: NARISU KESHOHIN KK (NARI-N)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 11343497	A	19991214	JP 98188008	A	19980529	200009	B

Priority Applications (No Type Date): JP 98188008 A 19980529
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 11343497	A		1	C11B-009/00	

Abstract (Basic): JP 11343497 A

NOVELTY - An aromatic composition, comprising flavour of rosemary, lavender, bitter orange or Senkyu.

DETAILED DESCRIPTION - Extract of the plant is incorporated as flavour ingredient.

USE - Useful as cosmetics showing sedative and hypnotic effect.

Dwg.0/0

Derwent Class: D21; D23
International Patent Class (Main): C11B-009/00
International Patent Class (Additional): A61K-007/00; A61K-007/46; A61K-007/48; A61K-035/78

8/7/5

009062710
WPI Acc No: 1992-190102/199223
New sleep promoting agent - comprises bitter orange essential oil, formulated for absorption through the nasal mucosa or the lungs
Patent Assignee: SUNTORY LTD (SUNR)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 4128234	A	19920428	JP 90249716	A	19900919	199223	B

Priority Applications (No Type Date): JP 90249716 A 19900919
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 4128234	A		7	A61K-035/78	

Abstract (Basic): JP 4128234 A

Pharmaceutical agent comprises bitter orange essential oil as an effective ingredient and is formulated to be absorbed through the nasal mucsa, the mouth mucosa or the lungs. The agent can be formulated as gum, candies, troches, soft drinks, juice, tea, jelly, milky lotion, ointment, lotion, spray, fragrance etc.

USE/ADVANTAGE - Useful for promoting sleepiness of those who cannot sufficiently sleep by stress etc. By dividing human subjects into a bitter orange essential oil treatment gp. and a control, giving them a light calculation work before sleeping and measuring each sleeping stage by his ECG and electro-encephalography. The result was that a time to attain sleepiness is significantly reduced for the treatment gp. (bitter orange conc. 0.2-0.5 ng/l.).

In an example, a mixt. of 20 pts. wt. gum base, 2 pts. wt. $CaCO_3$, 0.1 pt. wt. of stebiocide, 1 pt. wt. of bitter orange essential oil, 76.895 pts. wt. lactose and 0.005 pts. wt. fragrance was formulated to produce a gum prepn

Dwg.0/0

Derwent Class: B04
International Patent Class (Main): A61K-035/78
International Patent Class (Additional): A61K-009/06; A61K-009/08; A61K-009/107; A61K-009/12; A61K-009/20; A61K-009/68; C11B-009/00
?LOGOFF

8/7/3
DIALOG(R)File 352:Derwent WPI

009809424
WPI Acc No: 1994-089279/199411
Sleep promoter contg. Jasmine lactone as active component - is in form of preparation where active component is absorbed from lung or nose or mouth mucosa
Patent Assignee: SUNTORY LTD (SUNR)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 6040911	A	19940215	JP 92195333	A	19920722	199411	B

Priority Applications (No Type Date): JP 92195333 A 19920722
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 6040911	A		7	A61K-031/365	

Abstract (Basic): JP 6040911 A

The sleep promoter contg. jasmine lactone as the active component is made in a preparation such that the active component can be absorbed from the nose mucosa, the mouth mucosa or the lung.

USE/ADVANTAGE - Promoter can promote sleep advantageously.

In an example, 14 panellists were put in an electromagnetically shielded room held at 24 deg. C and 60% RH and their brain waves were recorded in a closed-eye rest period and in an open-eye rest period each for 3 minutes. Then, a calculating operation was performed by using a personal computer for 20 minutes. Then, a simple vision distinction test was made by red/blue colour reaction for 40 minutes. Jasmine lactone was diffused into the air to a concn. of 1.5 ng/l. The jasmine lactone dosed gp. showed significantly higher values of the powers of alpha wave and theta wave, compared to the control gp. with no dose. A chewing gum was prepd. by mixing 20 pts. of gum base, 2 pts. of Ca carbonate, 0.1 pt. of steviosite, 1 pt. of jasmine lactone, 76.895 pts. of lactose and 0.005 pt. of perfume.

Dwg. 0/3

Derwent Class: B02
International Patent Class (Main): A61K-031/365
International Patent Class (Additional): A61K-007/00; A61K-009/72; A61K-035/78

8/7/4
DIALOG(R)File 352:Derwent WPI

009655131
WPI Acc No: 1993-348682/199344
Perfume contg. sedative essential oil - has durable sedative effect
Patent Assignee: KANEBO LTD (KANE)
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
JP 5255688	A	19931005	JP 9289811	A	19920313	199344	B

Priority Applications (No Type Date): JP 9289811 A 19920313
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
JP 5255688	A		5	C11B-009/00	

Abstract (Basic): JP 5255688 A
A new perfume contg. a sedative essential oil is prepd. by removing high-temp. ingredients from cedarwood oil. The fraction of the retention time of 0-50 min. in the chromatogram of chromatography operated under the conditions; Equipment: Hewlett Packard Sha 5890A; Column: J&W Sha DB-WAX, 0.25mm ID x 60m length; Carrier gas: helium; Carrier gas flow: 1ml/min; Column temp.: 70-200 deg. C; Programmed rate: 2 deg. C/min; and Detector: hydrogen ionisaton detector, F.I.

The cedarwood is typically Cedrusiibani Barr., Cedrus atlantica Manetti and/or Cedrus deodara Loud. The oil is usually extracted with an organic solvent(s), such as petroleum ether or hexane. The retention time in the chromatogram is pref. 0-30 min. Holding agents are opt. added, including trimethyl citrate, tributyl citrate and/or benzyl benzoate.

USE/ADVANTAGE - The perfume has a high, long lasting sedative action.

Dwg.0/1
Derwent Class: D23
International Patent Class (Additional): A61K-007/46; C11B-009/02

011751958
WPI Acc No: 1998-168868/199815
Cosmetological method for achieving valuable cosmetic effects easily by massage - comprising massage carried out by ordinary people along arterial and then venous blood flow paths using cosmetic containing disintegrative particles
Patent Assignee: KAO CORP (KAOS)
Inventor: MINAMI T; NAGASHIMA Y; YADA Y
Number of Countries: 019 Number of Patents: 006
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9807403	A1	19980226	WO 97JP2902	A	19970821	199815	B
JP 10087431	A	19980407	JP 96261346	A	19960909	199824	
JP 10113369	A	19980506	JP 9770225	A	19970324	199828	
JP 10113370	A	19980506	JP 9770226	A	19970324	199828	
EP 872228	A1	19981021	EP 97935845	A	19970821	199846	
			WO 97JP2902	A	19970821		
US 6269817	B1	20010807	WO 97JP2902	A	19970821	200147	
			US 9851489	A	19980921		

Priority Applications (No Type Date): JP 9770226 A 19970324; JP 96239868 A 19960821; JP 96239869 A 19960821; JP 96261346 A 19960909; JP 9770225 A 19970324
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9807403	A1	J	71	A61H-007/00	
Designated States (National): US					
Designated States (Regional): AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
JP 10087431	A		12	A61K-007/00	
JP 10113369	A		12	A61H-007/00	
JP 10113370	A		11	A61H-007/00	
EP 872228	A1	E		A61H-007/00	Based on patent WO 9807403
Designated States (Regional): DE FR GB					
US 6269817	B1			A61H-023/06	Based on patent WO 9807403

Abstract (Basic): WO 9807403 A

A method for achieving cosmetic effects comprises massaging skin along the arterial blood flow and then along the venous blood flow paths.

Preferably the method comprises applying cosmetics to a part of the skin and then massaging. The massaged part can be the body or face. Massage is conducted along a direction from the end of the mouth to the wing of the nose and then along a circle on the cheek from the end of the mouth towards the ear via the lower eyelid. Another cosmetic massaging comprises (a) along a direction from the end of the mouth to the wing of the nose, (b) along a circle on the cheek from the end of the mouth towards the ear via the lower eyelid, (c) along circles from the middle of the forehead toward the ends of the forehead via the upper forehead, and (d) along the lower eyelids from one end of the eyes to the other.

USE - The method is easily achieved for general skin care by ordinary people. The method can be effectively applied to a person only when the pulse, skin vessel, skin temperature or skin blood flows are in an accelerated state.

ADVANTAGE - The method is easy and effective for cosmetic skin care.

Dwg. 0/14

Derwent Class: A96; D21; P33
International Patent Class (Main): A61H-007/00; A61H-023/06; A61K-007/00
International Patent Class (Additional): A61K-007/48; A61K-007/50; A61K-035/50; A61K-035/70; A61K-035/78

Title

Cardiovascular neural regulation explored in the frequency domain.

Author

Malliani A; Pagani M; Lombardi F; Cerutti S

Organization

Istituto Ricerche Cardiovascolari, Centro Ricerche Cardiovascolari, CNR, Milano, Italy.

Publication Source

CIRCULATION, **(1991 Aug)** 84 (2) 482-92. Ref: 107
Journal code: 0147763. ISSN: 0009-7322.

Document Type

Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

Language

English

Abstract

A consistent link appears to exist between predominance of vagal or sympathetic activity and predominance of HF or LF oscillations, respectively: RR variability contains both of these rhythms, and their relative powers appear to subserve a reciprocal relation like that commonly found in sympathovagal balance. In this respect, it is our opinion that rhythms and neural components always interact, just like flexor and extensor tones or excitatory and inhibitory cardiovascular reflexes, and that it is misleading to separately consider vagal and sympathetic modulations of heart rate. In humans and experimental animals, functional states likely to be accompanied by an increased sympathetic activity are characterized by a shift of the LF-HF balance in favor of the LF component; the opposite occurs during presumed increases in vagal activity. In addition, LF oscillation evaluated from SAP variability appears to be a convenient marker of the sympathetic modulation of vasomotor activity. Although based on indirect markers, the exploration in the frequency domain of cardiovascular neural regulation might disclose a unitary vision hard to reach through the assemblage of more specific but fragmented pieces of information.

Accession Number

91316808 MEDLINE

Title

Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog.

Author

Pagani M; Lombardi F; Guzzetti S; Rimoldi O; Furlan R; Pizzinelli P; Sandrone G; Malfatto G; Dell'Orto S; Piccaluga E; +

Publication Source

CIRCULATION RESEARCH, **(1986 Aug)** 59 (2) 178-93.
Journal code: **0047103**. ISSN: **0009-7330**.

Document Type

Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

In 57 normal subjects (age 20-60 years), we analyzed the spontaneous beat-to-beat oscillation in R-R interval during control recumbent position, 90 degrees upright tilt, controlled respiration (n = 16) and acute (n = 10) and chronic (n = 12) beta-adrenergic receptor blockade. Automatic computer analysis provided the autoregressive power spectral density, as well as the number and relative power of the individual components. The power spectral density of R-R interval variability contained two major components in power, a high frequency at approximately 0.25 Hz and a low frequency at approximately 0.1 Hz, with a normalized low frequency:high frequency ratio of 3.6 +/- 0.7. With tilt, the low-frequency component became largely predominant (90 +/- 1%) with a low frequency:high frequency ratio of 21 +/- 4. Acute beta-adrenergic receptor blockade (0.2 mg/kg IV propranolol) increased variance at rest and markedly blunted the increase in low frequency and low frequency:high frequency ratio induced by tilt. Chronic beta-adrenergic receptor blockade (0.6 mg/kg p.o. propranolol, t.i.d.), in addition, reduced low frequency and increased high frequency at rest, while limiting the low frequency:high frequency ratio increase produced by tilt. Controlled respiration produced at rest a marked increase in the high-frequency component, with a reduction of the low-frequency component and of the low frequency:high frequency ratio (0.7 +/- 0.1); during tilt, the increase in the low frequency:high frequency ratio (8.3 +/- 1.6) was significantly smaller. In seven additional subjects in whom direct high-fidelity arterial pressure was recorded, simultaneous R-R interval and arterial pressure variabilities were examined at rest and during tilt. Also, the power spectral density of arterial pressure variability contained two major components, with a relative low frequency:high frequency ratio at rest of 2.8 +/- 0.7, which became 17 +/- 5 with tilt. These power spectral density components were numerically similar to those observed in R-R variability. Thus, invasive and noninvasive studies provided similar results. More direct information on the role of cardiac sympathetic nerves on R-R and arterial pressure variabilities was derived from a group of experiments in conscious dogs before and after bilateral stellectomy. Under control conditions, high frequency was predominant and low frequency was very small or absent, owing to a predominant vagal tone. During a 9% decrease in arterial pressure obtained with IV nitroglycerin, there was a marked increase in low frequency, as a result of reflex sympathetic activation.(ABSTRACT TRUNCATED AT 400 WORDS)

Accession Number

86298915 MEDLINE

Title

Heart rate, heart rate variability, and **blood pressure** during perioperative stressor events in abdominal surgery.

Author

Schubert A; Palazzolo J A; Brum J M; Ribeiro M P; Tan M

Organization

Department of General Anesthesiology, Cleveland Clinic Foundation, OH 44195, USA.

Publication Source

JOURNAL OF CLINICAL ANESTHESIA, (1997 Feb) 9 (1) 52-60.
Journal code: 8812166. ISSN: 0952-8180.

Document Type

(CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)

Language

English

Abstract

STUDY OBJECTIVE: To define the behavior of power spectral heart rate variability (PSHR) during potentially stressful events in the perioperative period, and relate it to changes in **blood pressure** (BP) and heart rate (HR). DESIGN: Longitudinal clinical study. SETTING: Operating room and recovery suites of a large tertiary care referral center. PATIENTS: 26 ASA physical status I, II, and III patients undergoing elective abdominal surgery. INTERVENTIONS: Anesthesia was induced with thiopental sodium and fentanyl, and maintained with isoflurane/nitrous oxide (N2O)/**relaxant** or enflurane/N2O/**relaxant**. The trachea was intubated and intraabdominal surgery was performed. MEASUREMENTS AND MAIN RESULTS: Observations consisted of HR, noninvasive **blood pressure**, and PSHR. They were made before and after induction of anesthesia, tracheal intubation, and surgical incision, and during maximal surgical stimulation and skin closure. HR and mean arterial pressure (MAP) maxima were also recorded for one hour before and after emergence from anesthesia. PSHR was obtained using a special algorithm and data acquisition system for real time **spectral analysis** of the instantaneous HRversus time function. The HR power spectrum parameters analyzed were low-frequency (LFA; powerband = 0.04 to 0.10 Hz), respiratory-induced frequency (RFA; powerband = respiratory frequency +/- 0.06 Hz), and the ratio of LFA to RFA. With induction of anesthesia, only RFA power decreased significantly. LFA power reduction became significant only after intubation and continued so until after incision. Immediately after induction, the decline in RFA power (vs. preinduction) was more pronounced when compared with the decline in LFA power (76% vs. 34%; $p = 0.01$). Hence, the ratio LFA/RFA increased significantly after induction of anesthesia. It was significantly higher than at postintubation, preincision, or skin closure. A significant elevation in LFA, LFA/RFA ratio, and BP occurred with maximal abdominal surgical stimulation. Only preinduction LFA, RFA, and LFA/ RFA ratio were predictive of MAP changes with induction of anesthesia ($p = 0.006$). In 8 of the 15 patients who had MAP changes of at least 10 mmHg with induction, PSHR indices correctly predicted a change of this magnitude. Late intraoperative HR maxima were positively correlated with the change in HR and incision ($r2 = 0.58$; $p < 0.01$). The change in BP with incision was positively correlated with early postoperative HR maxima ($r2 = 0.60$; $p < 0.01$). CONCLUSIONS: On anesthetic induction, preoperative, but not intraoperative, spectral indices were predictive of BP changes. Power **spectral analysis** of HR mav provide information about the autonomic state that is not evident from BP

Research Advances Series

Cardiovascular Neural Regulation Explored in the Frequency Domain

Alberto Malliani, MD; Massimo Pagani, MD; Federico Lombardi, MD; and Sergio Cerutti, MS

This article discusses the clinical application and potentiality of a relatively new methodology, which in large part uses noninvasive recordings and appears to provide a quantitative evaluation of the sympathovagal interaction modulating cardiovascular function.

As a result of this methodology, pathophysiological conditions of paramount importance, such as arterial hypertension, myocardial ischemia, sudden cardiac death, and heart failure, for which the promoting or aggravating role of neural factors is still largely unknown, might soon undergo a novel scrutiny with practical implications.

Physiological Background

In addition to cardiac cycle, two main rhythmic events affect the circulation: respiration and vasomotion. The respiratory activity has long been known to be accompanied by arterial pressure[1] and heart period fluctuations, whereas the finding of slow arterial pressure oscillations (also referred to as Mayer waves), having a period of approximately 10 seconds, has been more elusive.[2-4] On the other hand, rhythmic discharges in phase with respiration have been described in the sympathetic[5] and vagal[6,7] outflows; similarly, a slower rhythm in phase with vasomotor waves has been found in the sympathetic[8,9] and vagal[10] efferent discharges.

The neural regulation of circulatory function is mainly effected through the interplay of the sympathetic and vagal outflows. In most physiological conditions, the activation of either outflow is accompanied by the inhibition of the other. The sympathovagal balance is tonically and phasically modulated by the interaction of at least three major factors: central neural integration, peripheral inhibitory reflex mechanisms (with negative feedback characteristics), and peripheral excitatory reflex mechanisms (with positive feedback characteristics)[11-13] (Figure 1).

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Address for correspondence: Alberto Malliani, MD, Medicina Interna, Ospedale "L. Sacco," Via G.B. Grassi 74, 20157 Milano, Italy.

It is the core hypothesis of the proposed approach that this balance, viewed as a reciprocal relation, can on the whole be explored in the frequency domain. That is, variable phenomena such as heart rate and arterial blood pressure can be described not only as a function of time (i.e., in the time domain), but they can also be described as the sum of elementary oscillatory components, defined by their frequency and amplitude. We review data that support the assumptions that 1) the respiratory rhythm of heart period variability, defined as the high-frequency (HF) spectral component, is a marker of vagal modulation; 2) the rhythm corresponding to vasomotor waves and present in heart period and arterial pressure variabilities, defined as the low-frequency (LF) component, is a marker of sympathetic modulation; and 3) a reciprocal relation exists between these two rhythms that is similar to that characterizing the sympathovagal balance.

Methodology

Figure 2 schematically illustrates the procedure of spectral analysis of heart period variability as performed in our laboratory. A similar procedure is used for other signals, such as arterial pressure (Figures 3 and 4), respiration, or nerve discharge (Figure 5). From the original electrocardiographic signal, a digital computer stores the time intervals between consecutive peaks of the R waves as the tachogram. In principle, the subsequent spectral analysis, used to detect possible rhythmicity hidden in the signal, necessitates stationary conditions that, in strict terms, are unknown to biology. Thus, a practical compromise has to be found between the length of event series and theoretical mathematical requirements.[14] Under adequate stationary conditions, the tachogram is not accompanied by slow trends or step changes (see Figure 2).

Various algorithms[15] can be used at this stage to assess the number, frequency, and amplitude of the oscillatory components. Most studies have relied on either the fast Fourier transform algorithm[16-19] or an autoregressive approach.[20-22] The former is easy to implement but requires strict periodicity of the data and is frequently used with an a priori selection of the number and frequency range of oscillatory components. Autoregressive algorithms (e.g., Figure 2)

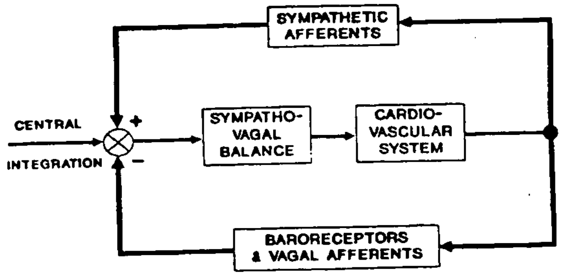

FIGURE 1. *Schematic representation of opposing feedback mechanisms that, in addition to central integration, subserve neural control of the cardiovascular system. Baroreceptive and vagal afferent fibers from the cardiopulmonary region mediate negative feedback mechanisms (exciting the vagal outflow and inhibiting the sympathetic outflow), whereas positive feedback mechanisms are mediated by sympathetic afferent fibers (exciting the sympathetic outflow and inhibiting the vagal outflow).*

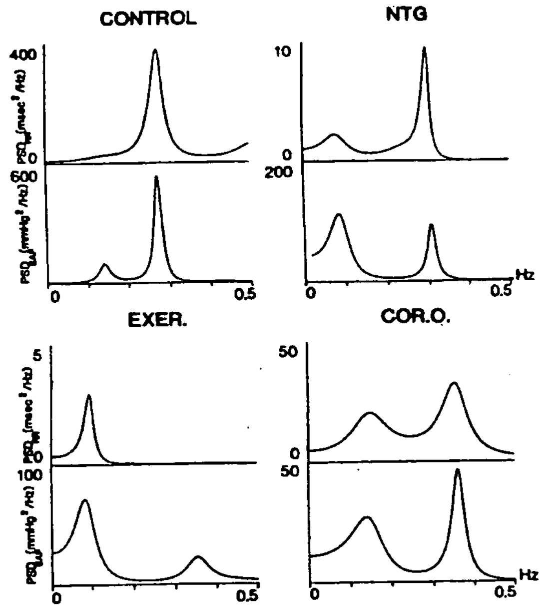

FIGURE 3. *Spectral analysis of RR interval (upper tracings in each panel) and systolic arterial pressure (SAP) (lower tracings in each panel) variabilities in conscious dogs at rest (CONTROL) and during experimental maneuvers leading to a sympathetic predominance (i.e., nitroglycerin infusion [NTG], treadmill exercise [EXER.], and transient acute coronary artery occlusion [COR.O.]). Note at control the presence of a single major high-frequency component in the RR interval autospectrum; in SAP, a smaller low-frequency component is also evident. During sympathetic activation, spectral distribution is altered in favor of low frequency; simultaneously, a drastic reduction in RR variance occurs (notice different scales on ordinates). PSD, power spectral density. From References 30 and 107 and unpublished material.*

can automatically furnish the number, amplitude, and center frequency of the oscillatory components without requiring a priori decisions. Because short segments of data are more likely to be stationary, the autoregressive algorithms, which are capable of operating efficiently even on shorter series of events, appear to provide an additional advantage.

The spectrum of Figure 2 contains three components, with frequencies centered at 0.00 Hz (component 1), 0.12 Hz (component 2), and 0.27 Hz (component 3), respectively. The study of the very low frequency (0–0.03 Hz) phenomena (component 1), which might contain clinically relevant information, requires specific methodologies and long periods of uninterrupted data.[23,24] Thus, component 1, considered DC, is not addressed in the present methodology. Components 2 and 3, labeled LF and HF, respectively, are evaluated in terms of frequency

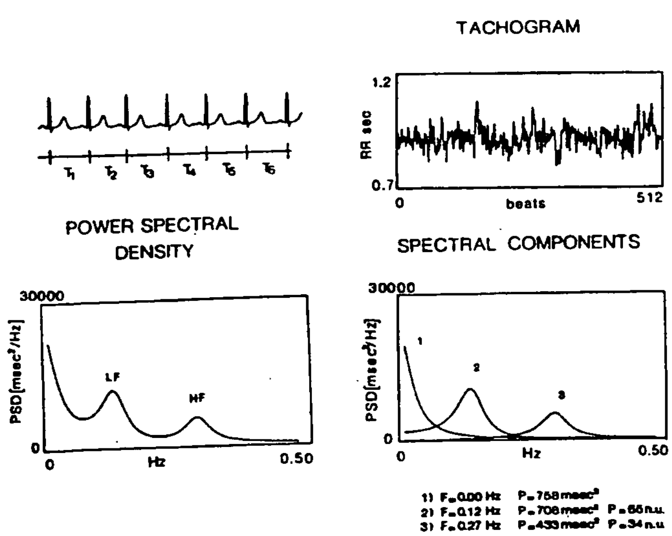

FIGURE 2. *Schematic representation of the method used for the spectral analysis of RR interval variability. From the surface electrocardiogram* (top left panel), *the program computes the individual RR intervals (T_1–T_6) and stores them in the memory as the tachogram. From the tachogram, power spectral density (PSD) is computed. Two major components, low frequency (component 2) and high frequency (component 3), are usually recognized as well as a large and variable fraction of very slow oscillations (below 0.03 Hz, component 1), which is not considered in the analysis. Note that the computer program automatically recognizes and prints out for each component the center frequency (F) and associated power (P) in absolute (msec²) and normalized units (n.u.) (see values in* lower right panel). *In the ordinates of lower panels, PSD units are in msec²/Hz; consequently, their integrated value corresponding to the area (i.e., power, obtained over any given frequency range in Hz) is expressed in msec². Reproduced with permission from Reference 26.*

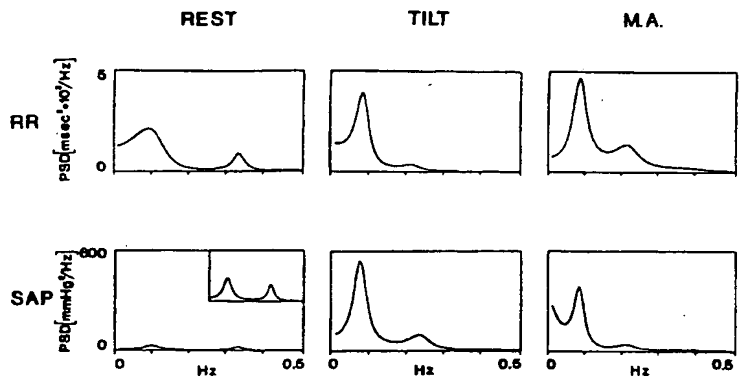

FIGURE 4. *Spectral analysis of both RR interval and systolic arterial pressure (SAP) variabilities in a human subject at rest, during passive tilt, and during a mental arithmetic test (M.A.). Note the presence at rest of two major spectral components in both RR and SAP variabilities (in SAP variability, a ×10 magnified spectrum is provided in inset). During tilt and M.A., there is a marked predominance of low-frequency component in both RR and SAP autospectra. PSD, power spectral density. From Reference 26 and unpublished material.*

(hertz in the figures) and amplitude. This amplitude is assessed by the area (i.e., power) of each component; therefore, squared units are used for the absolute value (milliseconds squared in Figure 2). In addition, normalized units (NU) are obtained by dividing the power of a given component by the total variance (from which component 1 has been subtracted) and multiplying by 100 (Figure 2). LF and HF components can also be found in the spectra of systolic arterial pressure (SAP) variability[22,25,26] (Figures 3 and 4) and of sympathetic and vagal efferent nerve discharges[27] (Figure 5). A recursive version of this methodology permits the analysis of recordings over a 24-hour period.[25,26,28,29]

Like the HF respiratory component, LF oscillation does not have a fixed period, and its center frequency can vary considerably (from 0.04 to 0.13 Hz).[26,30] Therefore, the convention of subdividing the low part of the spectrum into two preselected bands[16–18] with a cutoff frequency of 0.07–0.09 Hz[28,29,31] contained within the range of LF component appears unjustified.

Finally, it should be mentioned that from the simultaneous spectral analysis of RR interval and SAP variabilities,[19,32] a quantitative assessment of the overall gain of the baroreceptor mechanisms can be obtained.[33–35] In our studies,[25,33] this gain is represented by the index (α), which can be computed in correspondence to either LF or HF components. Its numerical value is provided by the square root of the ratio of the powers of RR to corresponding SAP spectral components.[25] In dynamic conditions, arterial pressure should be recorded with high-fidelity techniques,[25,26,36] whereas, in resting conditions, measurements with standard catheter-manometer systems[25,35] or noninvasive plethysmographic devices[19] can be adequate.

Comparable results were obtained[25] when the gain of the baroreceptor mechanisms was evaluated with both the index (α) and the phenylephrine method,[37] which is based on the slope of the reflex bradycardia accompanying a transient arterial pressure rise induced by intravenous injection of a pressor drug.

Animal Studies

A dominant role of the vagi in determining the HF component of RR variability was inferred from experiments in acute decerebrate cats[38] and conscious

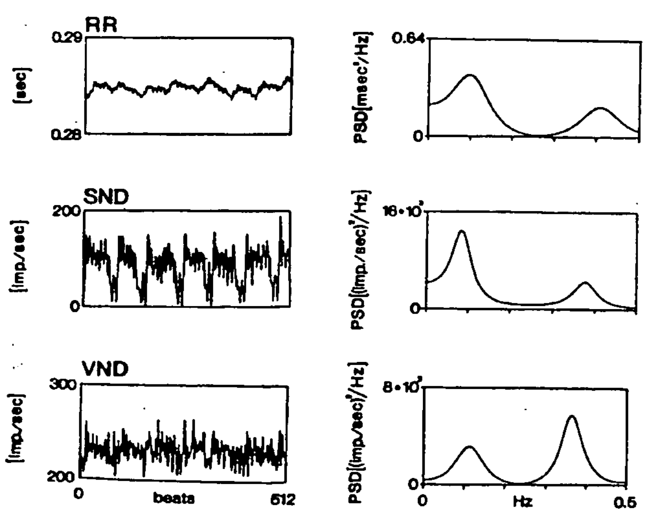

FIGURE 5. *Spectral analysis of RR interval, preganglionic sympathetic neural discharge (SND) recorded from third left thoracic sympathetic ramus communicans, and efferent vagal neural discharge (VND) simultaneously recorded from left cervical vagus in an artificially ventilated decerebrate cat. Time series of the three signals are illustrated on* left panels, *whereas their autospectra are represented on* right panels. *A predominant low frequency characterizes RR and SND autospectra, whereas a greater respiratory high-frequency component is present in VND variability. PSD, power spectral density. From Reference 27 and unpublished material.*

dogs[18,30,39,40] describing the effects of vagotomy and muscarinic receptor blockade.

Recently, Rimoldi et al[30] reported that in resting conscious dogs characterized by a marked HF predominance (Figure 3) resulting from high vagal tone,[41,42] a small LF component was always present in SAP variability but was present in only 50% of the cases in RR variability. However, an important finding was that whenever sympathetic excitation occurred, such as during baroreceptor unloading with nitroglycerin infusion, transient coronary artery occlusion, or physical exercise (Figure 3), a significant increase in LF was observed. The role played by baroreceptive mechanisms in these various experimental conditions was probably different because arterial pressure was reduced by nitroglycerin infusion, unchanged during myocardial ischemia, and increased with exercise. Therefore, LF component should not be considered a specific reflection of a baroreflex compensatory response[43] but rather a general marker of sympathetic excitation, regardless of its mechanism.

During muscarinic receptor blockade,[30] which drastically reduced total RR variance, all of the remaining power in control conditions and during baroreceptor unloading was concentrated in the LF region, in accordance with the sympathetic predominance induced by the drug. Finally, after chronic bilateral stellectomy producing cardiac sympathetic denervation, baroreceptor unloading no longer induced an increase in the LF component of RR variability. On the contrary, the increase in the LF component of SAP variability was still present.[22,30]

It was inferred that in RR variability, HF was mainly mediated by vagal mechanisms, whereas the sympathetic outflow appeared essential to the LF increases. Furthermore, the importance of neural mechanisms in mediating LF and HF of RR variability and LF of SAP variability was proven[30] by their disappearance during ganglionic transmission blockade obtained with intravenous infusion of trimethaphan.

To conclude on the most relevant results from animal experiments, which appear to validate the proposed approach, we emphasize that both LF and HF components can be directly and simultaneously detected from the sympathetic and vagal efferent impulse activities. In the example of Figure 5, the spectral analysis of RR, sympathetic, and vagal variabilities reveals corresponding LF and HF components, with a predominance of LF in the sympathetic discharge and of HF in the vagal activity. This fact, on the one hand, stresses that spectral analysis can be used to examine the complexities of neural regulation without artificially isolating the influence of either outflow and, on the other hand, suggests that a rhythm, being a flexible and dynamic property of neural networks,[44,45] should not necessarily be restricted to one specific neural pathway to carry a functional significance.

Human Physiological Studies

Use of LF and HF Components to Assess Sympathovagal Balance

The total power of RR interval variability (i.e., variance) has been interpreted as a selective index of cardiac parasympathetic tone[46,47]: however, in conditions characterized by an augmented sympathetic activity, it does not always appear to be capable of reflecting the balance with the concomitant vagal withdrawal.[22,48–50]

In the resting normal subject, power spectral analysis reveals two main rhythmic oscillations in heart period and arterial pressure variabilities[22,25] (Figures 2 and 4). LF component usually has a center frequency of approximately 0.1 Hz (0.12 Hz in Figure 2), whereas HF component, synchronous with respiration, occurs at approximately 0.25 Hz (0.27 Hz in Figure 2). The power of the LF component is greater than that of HF in RR variability with an LF-to-HF ratio of usually more than 1.[22,48,49]

Effects on RR Variability of Maneuvers Enhancing Sympathetic Drive

Passive tilt or, more simply, standing up is accompanied by an increase in the LF and a decrease in the HF component of RR variability (Figure 4).[20,22,48,49,51–54] The LF-to-HF ratio is greatly enhanced, to values as great as 20 in young subjects.[22]

Mental stress induced by arithmetic calculation has been shown to enhance sympathetic activity and alter the sympathovagal balance. This is reflected by a reduction in total power,[55,56] an increase in LF, and a decrease in HF (Figure 4).[57,58]

Physical exercise increases sympathetic activity and is associated with various factors such as enhanced respiratory activity, decreased variance, and increased non stationarity, all of which might contribute to a difficult analysis. Although Pagani et al[25] described, for mild levels of exercise, a clear predominance of the LF component in RR variability, this phenomenon has been negated by Arai et al.[59] In general, however, when a sympathetic activation is accompanied by an abatement of RR variance, as takes place physiologically during physical exercise, pharmacologically after atropine administration, or in various pathophysiological conditions, it is crucial to peruse where the residual power is distributed: The state of the balance would still be reflected by the relation between LF and HF components.

Effects on RR Variability of Maneuvers Enhancing Vagal Drive

A nonlinear relation exists between respiration and sinus arrhythmia[60]; however, controlled respiration at frequencies within the resting physiological range[60] provides a convenient tool to enhance the vagal modulation of heart rate,[22,51] probably also achieved through the synchronization of all respiratory components. In concrete terms, the power of the HF component becomes predominant at rest during

metronome breathing, leading to an LF-to-HF ratio of less than 1.[22] Furthermore, during controlled respiration, increases in the LF component and LF-to-HF ratio observed with tilt are markedly blunted in regard to that obtained during spontaneous respiration.[22] If the frequency of controlled breathing is decreased enough to approach LF rhythm, the two components merge into one more powerful oscillation.[60] In general, all of the studies that have been performed under controlled respiration in the broad range of 0.20–0.30 Hz were likely to be characterized by a sympathovagal balance shifted in favor of the vagal component.[51,61–63]

Effects on RR Variability of Aging

RR variance has been shown to decrease as age increases[22,64–67]; however, the LF-to-HF ratio, when measured with autoregressive algorithms, appears unchanged.[22] The increase in LF and the reciprocal decrease in HF of RR variability during tilt are also spared by aging, although they are blunted in their magnitude.[22] Changes in spectral components induced by standing were more difficult to determine in the elderly with a fast Fourier transform algorithm (see "Methodology"), probably as a consequence of the reduced variance and the low signal-to-noise ratio.[61,66,67]

Pharmacological Blockades, Neural Lesions, and RR Variability

From the observations by Selman et al[68] it became clear that atropine administration was capable of practically abolishing the respiratory component of RR variability; this finding was corroborated by the study of Pomeranz et al.[51] On the basis of these studies as well as animal studies, the relation between vagal activity and HF component of RR variability has become generally accepted.

However, there has been disagreement in the literature regarding the interpretation of the LF component. In the same study by Pomeranz et al,[51] intravenous administration of atropine in supine patients under controlled respiration was also capable of reducing the LF component by 84%; it was concluded that in this position, the LF component is mediated entirely by the parasympathetic system. However, because metronome breathing markedly enhances vagal drive and decreases the LF component,[22] this general statement is unlikely to be valid in the case of spontaneous respiration.

Furthermore, Inoue et al[69] noticed that in tetraplegic patients, the LF component was absent and HF was well preserved. They concluded that the absence of the LF component was likely to depend on the interruption of the spinal pathways connecting supraspinal centers with the peripheral sympathetic outflow.

Regarding the effects of β-adrenergic receptor blockade, Pagani et al[22] observed that although acute intravenous administration of propranolol blunted only the LF increase induced by tilt, as described by Pomeranz et al,[51] chronic oral administration significantly reduced the LF component (evaluated in NU) both at rest and during tilt. The fact that the LF component was clearly reduced but not abolished after chronic β-blockade, which differs with observations in tetraplegic patients,[69] might be a result of either the incompleteness of a pharmacological blockade in the clinical setting or a different basal contribution of vagal activity.

SAP Variability

The LF component has been reported to increase during tilt,[22,70] mental stress[57] (Figure 4), and physical exercise.[25,70]

During physical exercise, the analysis of SAP variability appears particularly suited to demonstrate an increased sympathetic drive because both its total variance and the LF component remain elevated, at least in correspondence with the mild levels of activity that have been examined so far.[25,26,30,70] On the other hand, in these conditions, the HF component of SAP variability is likely to depend mostly on mechanical effects of respiration,[26,30,71] because vagal modulation of RR interval with its resultant effects on stroke volume and arterial pressure should be nearly abolished during exercise.

Continuous 24-Hour Recording of RR and SAP Variabilities

Since initial observations,[72] a clear circadian oscillation has appeared to characterize the sympathovagal balance.[25] In more detail, the LF component of SAP variability increased abruptly with waking up[26] while the subjects were still lying in bed; remained elevated during the day,[26,29] especially in correspondence with physical exercise[26]; and then underwent a final marked reduction during the night.[26,29]

A similar circadian pattern in which LF and HF components of RR variability underwent reciprocal changes during the 24 hours could also be assessed with electrocardiographic Holter monitoring[26] (Figure 6, left panels). Conversely, in a study in which the LF range was subdivided into two predetermined bands of interest, separated arbitrarily at 0.07 Hz, and the heart period was derived from ambulatory arterial pressure recordings obtained with a system of narrow frequency response, Parati et al[29] did not detect in normal subjects the circadian pattern of LF component ("mid frequency" is the term used by the authors).

Human Pathophysiological Studies

Arterial Hypertension

It is an appealing hypothesis that essential hypertension, at least in its early stages, is largely based on increased sympathetic activity.[13,73] In a study[53] comparing hypertensive patients with normotensive age-matched controls, it was found that in RR variability, under resting conditions LF was greater (LF, 68±3 versus 54±3 NU) and HF was less (HF, 24±3 versus 33±2 NU) in hypertensive patients, suggesting an enhanced sympathetic activity and a reduced vagal activity. In hypertensive patients, passive tilt pro-

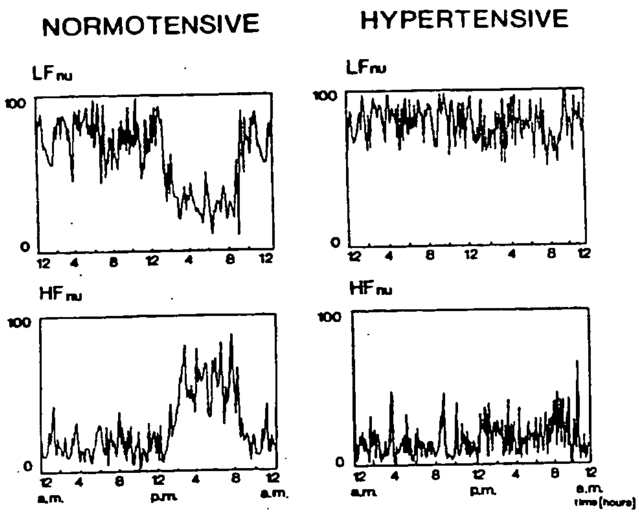

FIGURE 6. *Computer analysis plots of 24-hour RR interval variability (Holter recordings) in a normotensive subject (32-year-old man with 110/70 mm Hg resting arterial pressure) and a hypertensive subject (37-year-old woman with 160/105 mm Hg resting arterial pressure). Low-frequency (LF) and high-frequency (HF) components are represented in normalized units (nu). Note that a clear circadian cycle of both spectral components is present in only the normotensive subject. From Reference 74 and unpublished material.*

duced smaller increases in LF (Δ LF, 6.3±3 versus 26±3 NU) and decreases in HF (Δ HF, −7.5±2 versus −21.5±2 NU) than in normotensive controls. Furthermore, the values of LF at rest and the altered effects of tilt on LF and HF were significantly correlated with the degree of the hypertensive state, suggesting a continuum distribution.

When RR variability was studied throughout the 24-hour period with the use of Holter recordings, patients with essential hypertension were characterized by the loss of the circadian rhythmicity of the LF component (Figure 6, right panels), whereas a small nocturnal increase in HF was still detectable.[74] These data, although difficult to interpret, suggest that in hypertensive patients an increased sympathetic drive in basal conditions might be associated with a reduced responsiveness of neural regulatory mechanisms as assessed by spectral analysis.

In an invasive study[25] in normotensive and hypertensive subjects undergoing 24-hour continuous recording of electrocardiogram and arterial pressure measured with a high-fidelity technique, the overall gain of the baroreceptive mechanisms was evaluated with the index (α) (see "Methodology"). This index underwent a clear circadian variation, being smaller during the day, and was found to be decreased at rest in the hypertensive group, confirming that neural buffering mechanisms appear attenuated in essential hypertension.[75]

Ischemic Heart Disease

Experimental coronary artery occlusion can elicit neural and hemodynamic reflex responses that simultaneously include, from the heart, excitatory mechanisms mediated by cardiac sympathetic afferents[11,76,77] and inhibitory mechanisms mediated by cardiac vagal afferents.[12,77] In the clinical setting[78] of hyperacute phases of myocardial infarction, almost constant findings during the first 30 minutes after the onset of symptoms were signs of either sympathetic hyperactivity, which were more frequent in the course of anterior localization, or vagal hyperactivity, which were more frequent during inferior wall infarction. Such an "autonomic disturbance," assessed on the basis of heart rate and arterial pressure values, coincided with the highest incidence of life-threatening arrhythmias. In an ongoing study (Figure 7), we are finding that at about 1–3 hours after the onset of symptoms, spectral analysis of RR variability reveals a sympathetic predominance that is particularly evident in anterior wall localization.

In relation to survival after myocardial infarction, a quite simple analysis in the time domain of heart rate variability, such as that offered by the use of either standard deviation or variance, has recently provided important clinical information.[47,79–81] In particular, when applied on a large scale, a reduced standard deviation was found to carry a relevant prognostic value, being an independent predictor of mortality.[47] This reduction in standard deviation was attributed to a decreased vagal tone, which might also be reflected by a diminished total power of 24-hour spectral analysis,[82] leading to the hypothesis[47,80] of a simultaneous sympathetic predominance.

This hypothesis was fully supported by a study[48] in which we applied spectral techniques to analyze heart rate variability in a population of patients 2 weeks and 6 and 12 months after acute myocardial infarction. After 2 weeks, the LF component was significantly greater (69±2 versus 53±3 NU) and the HF component was significantly smaller (17±1 versus 35±3 NU) than in control subjects. This difference probably reflected an alteration of sympathovagal balance with a predominance of sympathetic activity. At 6 and 12 months, a progressive decrease in LF (62±2 and 54±3 NU) and increase in HF (23±2 and 30±2 NU) spectral components were observed, which suggested a normalization of sympathovagal interaction. Regarding the effects of tilt, 2 weeks

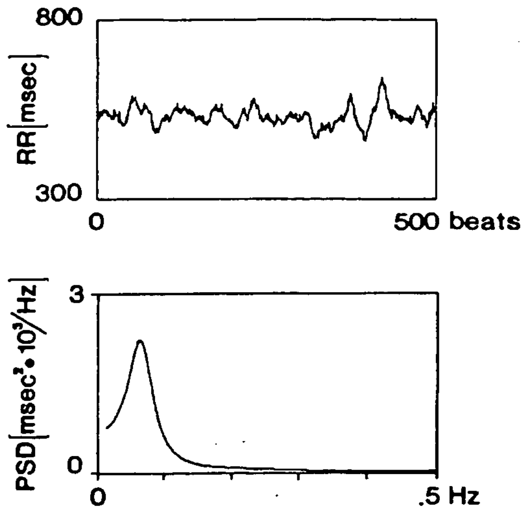

FIGURE 7. *Spectral analysis of RR variability in a patient (62-year-old man) with an acute anterior myocardial infarction, recorded within 1 hour from onset of symptoms. Note predominant low-frequency component, suggesting sympathetic overactivity. PSD, power spectral density.*

after myocardial infarction, this maneuver did not further modify the LF component of RR variability, whereas 1 year later, tilt was accompanied by an increase in the LF component of a magnitude similar to that observed in control subjects. One month after myocardial infarction, mental stress also failed to induce a significant increase in the already augmented LF component of RR variability.[58] More recently, the sympathetic predominance observed 2 weeks after myocardial infarction was studied during a 24-hour period and found to also persist at night, indicating that the normal circadian rhythm was markedly blunted.[83] The state of the sympathovagal balance in the period after the acute myocardial infarction has also been explored with the phenylephrine method,[84] and the results were compatible with the hypothesis of increased sympathetic and reduced vagal activities. All of these findings might provide a pathophysiological basis for the beneficial effects of β-adrenergic receptor blockade after myocardial infarction.

Regarding episodes of transient myocardial ischemia, as defined by electrocardiographic changes, in the limited number of patients reported by Bernardi et al[85] and in our investigation,[45] an increase in the LF component of RR variability was observed simultaneous with an increase in heart rate and independent of the occurrence of pain,[86] suggesting an excitatory reflex originating from the heart.[11,76,77]

Finally, a significant relation has recently been found between the extent of coronary artery disease and the amplitude of HF component in RR variability,[62] leading to the conclusion that a reduced cardiac vagal function might correlate with angiographic severity of coronary impairment.

Cardiac Transplantation

The condition after human heart transplantation represents a clinical model of denervated heart that has prompted various studies with spectral analysis.[87-89] In general, reduced total RR variance was found. However, although in some studies[87,89] no discrete HF or LF components were consistently found in RR variability, Bernardi et al[88] described a small HF component interpreted to be independent of neural mechanisms. Furthermore, in one patient studied by Fallen et al[87] 33 months after transplantation, both LF and HF components were present, the latter increased by synchronous respiration and abolished by atropine. They concluded that spectral analysis could offer a unique method for establishing the state of possible reinnervation of human transplanted heart. Finally, Sands et al[89] reported an increased variance in patients developing an allograft rejection, a finding, however, that has been challenged by others.[87,90]

Congestive Heart Failure

Congestive heart failure often appears to be accompanied by an increase in sympathetic activity.[91] Reduced RR variability has been observed in patients with chronic congestive heart failure[92,93] and interpreted as a sign of decreased parasympathetic activity. However, irrespective of its etiology, congestive heart failure can be characterized by various clinical manifestations, among which acute pulmonary congestion is likely to play a crucial role in determining the state of sympathovagal balance through a reflex enhancement of sympathetic activity.[94] In an ongoing study in patients with chronic congestive heart failure, we are finding that those in New York Heart Association functional class II or III are characterized by reduced variance and enhanced LF and reduced HF components, whereas a drastically diminished variance with only a residual HF component appears to be present in class IV patients. These preliminary observations suggest that signs of persistent sympathetic activation might be easier to recognize during the less advanced stages of the disease. On the other hand, in patients with severe chronic heart failure, Saul et al[92] observed only very low frequency spectral components, usually centered near 0.015 Hz, which they attributed to a preserved sympathetic modulation; this conclusion might deserve further appraisal because this part of the spectrum is markedly affected by slow trends and DC component.

However, despite these uncertainties, spectral analysis appears adequate to assess the changes of the sympathovagal balance throughout the various stages and types of this complex clinical condition, thus contributing to the information required by a rational therapy.

Chagas' Disease

Both reduced parasympathetic[95] control of heart rate and impaired sympathetic[96] responsiveness have been reported in chronic Chagas' disease. In patients with positive serology and electrocardiographic alterations usually found in this disease but without heart failure, RR variance and power spectral profile at rest were not different from those of controls[97,98]; however, when patients were standing, the usual increase in LF and decrease in HF were not present. This quantitative assessment of the altered neural modulation of heart rate might be useful in assessing the clinical progression of the disease.

Diabetic Neuropathy

Results of functional tests based on reflex cardiovascular responses have suggested a progressive deterioration of parasympathetic and, subsequently, sympathetic regulation in the course of diabetic visceral neuropathy.[99] Studies of RR variability indicated that diabetic patients have a reduced variance.[31,46,49,100] Furthermore, in a group of patients without overt clinical signs of neuropathy, the spectral profile was normal at rest. However, during tilt[49] or standing,[101] the increase in LF and decrease in HF components were markedly attenuated. This approach, which does not require that the patients engage in active tasks, like in the case of functional tests, could be even more appropriate for large-scale studies aimed at quantifying the early visceral neuropathy, its evolution, and possible therapies.

Future Lines of Research

Recent clinical investigation has clearly evidenced the more frequent occurrence of several types of acute cardiovascular events in the early morning hours,[102] which is when sympathetic activity undergoes a sudden surge.[26] This suggests that neural mechanisms might play a crucial triggering role. Accordingly, a field of extreme relevance in which more information is needed on the neural mechanisms involved concerns acute cardiovascular events and, in particular, the prevention of sudden cardiac death.[103-106] The inclusion of spectral analysis of cardiovascular variability in research protocols is now feasible and promising.

Summary

A consistent link appears to exist between predominance of vagal or sympathetic activity and predominance of HF or LF oscillations, respectively: RR variability contains both of these rhythms, and their relative powers appear to subserve a reciprocal relation like that commonly found in sympathovagal balance. In this respect, it is our opinion that rhythms and neural components always interact, just like flexor and extensor tones or excitatory and inhibitory cardiovascular reflexes, and that it is misleading to separately consider vagal and sympathetic modulations of heart rate. In humans and experimental animals, functional states likely to be accompanied by an increased sympathetic activity are characterized by a shift of the LF–HF balance in favor of the LF component; the opposite occurs during presumed increases in vagal activity. In addition, LF oscillation evaluated from SAP variability appears to be a convenient marker of the sympathetic modulation of vasomotor activity.

Although based on indirect markers, the exploration in the frequency domain of cardiovascular neural regulation might disclose a unitary vision hard to reach through the assemblage of more specific but fragmented pieces of information.

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Serial No. 09/972,887

In the matter of the Application of: Yoshinao NAGASHIMA, et al.

For: AUTONOMIC NERVE REGULATING AGENT

Due Date: N/A

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